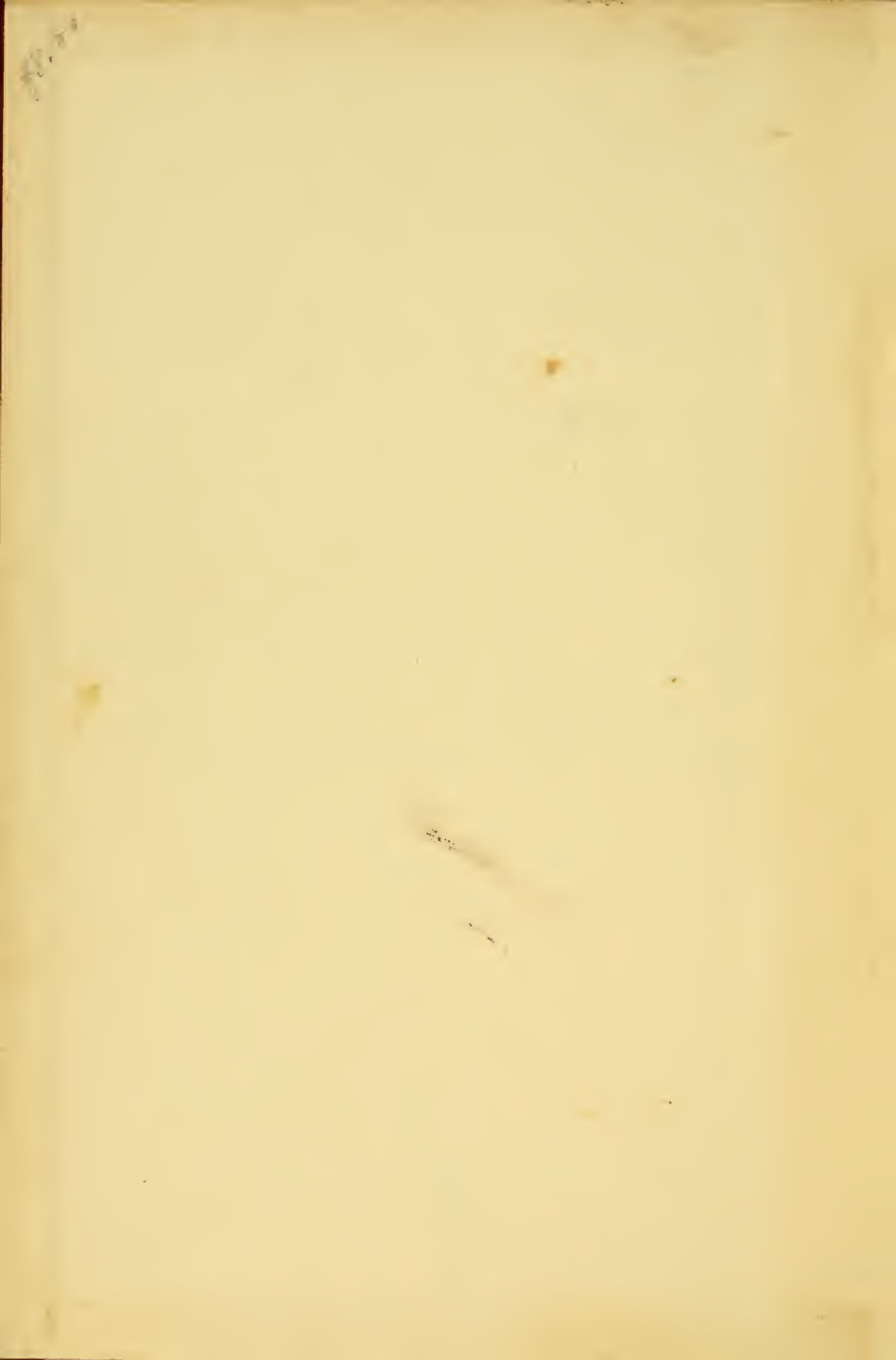
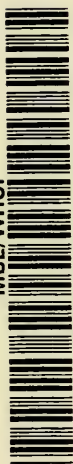




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PATTERNS AND PROBLEMS
OF DEVELOPMENT

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Patterns and Problems of Development

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PREFACE

DEVELOPMENTAL physiology is often regarded as if it were concerned only with embryonic development, and for some authors of recent years it seems to be very largely a matter of vertebrate or even of amphibian development. It cannot detract in any way from the great interest and importance of the experimental work of Spemann, his students, and many others on amphibian development to point out that this and development of vertebrates generally constitute only a small part and, so far as fundamental problems are concerned, probably not the most important part of the field of developmental physiology. Other patterns of embryonic development than those of amphibians or of other vertebrates are of equal or greater significance in relation to the physiological problems of development. Some of them appear to present the problem of developmental pattern in simpler, more general form than the vertebrates.

Moreover, the animal egg at the beginning of embryonic development gives little information concerning the beginnings and origins of developmental pattern. Pattern is already present in eggs—often advanced in development—when what we commonly call “development” begins, and the ovarian developmental period is at present almost inaccessible to analytic experiment. If we are to be consistent, we must admit that embryonic development in general is by no means the only material of developmental physiology. There are many other forms of development, with other starting-points than eggs. Some of them, such as buds, reconstructions of isolated pieces, and development of cell aggregates, bring us much nearer the beginnings of developmental patterns and the factors concerned in their origins and permit more extensive control and analysis than do most eggs and embryos. In fact, embryonic development appears generally to be the most highly specialized form of development. The animal egg is one of the most highly differentiated cells of the body. Only by comparison and analysis of all forms of development can we hope to distinguish the physiologically fundamental factors in developmental pattern from those which are incidental to a particular kind of development and to arrive at an adequate concept of the physiological foundations of development. The following pages, though very far from accomplishing

any such result, constitute in some degree an attempt at a comparative consideration and analysis of data concerning some of the various sorts of development and the characteristics of their patterns, with some discussion of what is known or believed or remains to be discovered concerning their origins. Embryonic development is considered not as the primary and fundamental form of development to which all other patterns are to be referred but rather as representing a relatively specialized type to be interpreted in the light of what we learn concerning other more primitive developmental patterns.

In certain species buds (often more than one kind of buds), isolated pieces of various sizes from various parts of the body, and even aggregates of dissociated cells may all give rise to individuals of the same sort as the individual developing from an egg. These different starting-points obviously do not all have the same pattern or organization as the egg, but we can scarcely avoid the conclusion that the essential factors of pattern must be similar in all. What are these factors, as distinguished from those incidental to a particular sort of development? To the writer this question appears to present a fundamental problem of developmental physiology. With it this book is largely concerned.

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A few citations of papers too recent to be included in the Bibliography and of some others which should have been included are given in footnotes or in the text.

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CHAPTER I

PROBLEMS AND MATERIAL

DEVELOPMENT of the individual organism is a continuous series of events in space and time. At any period of development the order is evident as a definite system of activities and structures which constitute the pattern of the individual at that period. The individual is a realization in these activities and structures of potentialities of the protoplasm concerned in relation to conditions external to it, and these external conditions are essential factors in determining what potentialities are realized in any particular case. When we subject developing organisms to experimental conditions, the course of development may be modified and the resulting organism may be widely different from that characteristic of the species under natural conditions. These modifications represent realization of other potentialities. Evidently the so-called "normal" individual is a realization of only a part of the potentialities of the species-protoplasm. Since development of different organisms takes place in protoplasts which differ specifically in constitution and possess different potentialities, the individual structural and functional patterns which develop also differ; and on the basis of these differences we distinguish species, genera, and larger groups.

The individual organism is a physiologically integrated unit—a whole with actual physiological relations between its parts, although at certain developmental stages a greater or less degree of independence of some parts as regards further differentiation may occur. In short, it seems necessary to conceive development as essentially a series of events integrated into a definite order, pattern, and unity which differs in character with the constitution of the protoplasm in which development is taking place. What problems does this phenomenon of development present? To ask this question is to ask what the problems of biology are, for life is development. And if we attempt to distinguish certain problems as fundamental, we are in no better case, for, unless we are content with the mere accumulation of facts, we cannot proceed far in the study of development from any point of attack without coming face to face with the great problems of biology.

Workers in the field of developmental physiology have attempted by many lines of experimental analysis to learn something more about development than can be learned from observation alone. Their experiments have not only given us a great body of data but have thrown much light on various aspects of development concerning which we could have learned little or nothing from mere observation, and have focused attention on various problems which promise to provide fruitful fields for further investigation.

To many biologists, even many students of developmental physiology, the word "development" means embryonic development. They recognize, of course, that other forms of development occur, but consider them of secondary importance. As a matter of fact, embryonic development is only one among many forms or types of development, and probably the most highly specialized of all. Many organisms, both plant and animal, develop in various ways which result either in similar or in different sorts of individuals. For example, hydroids may develop from buds, and the resulting individuals may be like the parent or different from it; they may also develop from isolated pieces of the mature body, or in some cases from aggregates of dissociated cells, as well as from eggs or parts of eggs, embryos, or larval forms. Again, the organization of the ascidian egg is evidently not essential for development of an ascidian, for an individual of the same sort may develop from an egg, from the tip of a stolon which itself originates as a bud, directly from a bud without stolon development, from cell aggregates formed from parts of an otherwise degenerating parent (the so-called "winter buds" of various forms), from experimentally isolated pieces of the mature individual, either by direct reconstitution or from cells of the piece remaining after partial degeneration, and from pieces of stolon. Unquestionably, something about the physiology of development of a hydroid or an ascidian is to be learned from all these different ways in which it occurs, perhaps even more from some of them than can be learned from the highly differentiated egg and its development alone. Plants develop from eggs and from buds of various sorts originating from sporophyte or gametophyte tissues, many of the simpler forms from naturally or experimentally isolated pieces or single cells of the plant body.

The occurrence of these many different forms or types of development raises at once the question whether they are fundamentally different in pattern or whether, in spite of the different starting-points, essential similarity of pattern is to be found in some or all of them. Unless we are willing to assume some metaphysical principle such as Driesch's entelechy

with power of free choice, underlying and guiding development, we should expect to find fundamental similarities of pattern in all of them. In any case, there is in all a definite developmental pattern involving spatial and chronological order and physiological integration. There is reason to believe that the view so widely current, either implicitly or explicitly, that embryonic development is of primary, and all other forms of development of secondary, importance, has in some measure led us astray in concentrating much of our attention on problems concerned with this form of development alone rather than on the problem of developmental pattern in general and the question what, if any, features of developmental pattern are common to all forms of development. By every criterion which we can apply, the oöcyte and the spermatozoon are highly specialized and differentiated cells. Evidently the differentiation of the egg cytoplasm is a factor in determining the course of development of the embryo: ascidian embryonic development is an excellent example. But, as noted above, an ascidian can develop in various other ways from other starting-points—from buds, pieces of body or stolon, etc., which certainly differ in organization from the egg and from each other. Embryonic development in a particular species under natural conditions always has the same starting-point, the egg, which always, except for genetic potentialities, has the same organization in a particular species. It seems beyond question that embryonic development is a relatively highly specialized form of development. If this is true, there is undoubtedly much to be learned concerning the fundamentals of developmental pattern from the bud, the piece, and other forms of development, as well as from the egg. May we not hope to attain a more adequate conception of development by including in our analysis other forms of development as well as egg and embryo? May it not even be possible to interpret certain features of embryogeny in the light of what we learn from other forms of development?

Any consideration of the physiology of development, if it is to be more than a mere compilation of data, must itself have an order and pattern and be integrated into a whole. The ordering and integrating factors in the following pages are the problems of pattern, order, and integration, particularly in the earlier stages of development.

THE PROBLEM OF ORGANIZATION

PROTOPLASM AND ORGANISM

A “protoplasm” is a complex physicochemical system differing in constitution in different species and, to some extent, in different individ-



uals; an "organism" is a system of protoplasts, an order and pattern apparently on a larger scale than that of a protoplasm. The most general characteristic of organismic pattern is a surface-interior difference, and in some simple organisms such difference apparently constitutes the only persistent organismic pattern, though other patterns may be temporarily superimposed on it. In *Amoeba*, for example, pattern is apparently primarily surface-interior; but the formation of a pseudopod alters and complicates the pattern temporarily, and in some cases the whole body may become temporarily an anteroposterior pattern with activity of a single pseudopodial region dominating it. Environmental factors are apparently directly concerned in the origin of surface-interior pattern. The surface of the protoplasm exposed to the external medium or in contact with other systems like itself, as in multicellular tissues, becomes different from the interior. The characteristics of the cell nucleus and its relations to the cytoplasm suggest that it originated as a differentiation of the interior. Nuclei or their parts may possess a very definite pattern, but this pattern is not the pattern of the organism or the cell and apparently cannot autonomously determine that pattern. At present there is no ground for believing that even surface-interior pattern can arise independently of environment.

AXIATE PATTERNS

In most organisms we find, in addition to surface-interior pattern, other orders or patterns which biologists have been accustomed to distinguish as polarity, radial and bilateral symmetry, ventrodorsality, dorsiventrality, and various asymmetry patterns. A polarity is a single serial order in a certain direction, referable to an imaginary polar axis. Symmetries and asymmetries may be regarded as polarities in other directions, referable to other axes—radial, ventrodorsal, dorsiventral, lateral—or in other directions in organ systems. On the other hand, polarity is an asymmetry in a certain direction. In other words, the different directions to which the spatial orders of organismic pattern are referable do not necessarily represent fundamentally different features of pattern. The terms "polarity," "symmetry," and "asymmetry" merely provide convenient distinctions for patterns in different directions in the developing organism, the term "polarity" usually being applied to the order which becomes evident first in development or is most conspicuous, with "symmetry" and "asymmetry" applied to secondary orders in other directions, usually becoming evident under natural conditions later in development than polarity. It

is also often convenient to conceive the direction in which these orders appear as representing a physiological axis and to designate developmental pattern in which serial orders of this sort occur as axiate pattern. Not only the whole organism but its various parts, appendages, many organs, or even individual cells may develop axiate pattern, and the axes of such patterns may be in all possible directions within the same organism.

The features of axiate pattern which first become evident in development are polarity and symmetry or asymmetry of the whole. These seem to constitute a sort of background in relation to which the further development of pattern takes place. They may be compared to a system of co-ordinates, a frame of reference, with respect to which each part has a definite position. This analogy, however, must not be pushed too far, for observation of the course of development indicates, and experimental analysis demonstrates, that the factors which constitute this apparent frame of reference are not merely formal in character but are physiologically operative in localization and determination of the course of development and that their action can be altered or they can be obliterated experimentally by change in physiological condition of the protoplasm concerned. They apparently constitute wholeness in its simplest, most general terms. They appear to be the primary organizing and also the primary integrating factors.

Polarities, symmetries, and asymmetries of one kind or another appear in many nonliving systems. In crystals, for example, we find one sort of polarity and symmetry; in flames, flowing streams, electric currents, etc., polarities of other kinds. Many biologists have attempted to interpret axiate pattern in organisms in terms of protoplasmic molecular or micellar orientation, symmetry, or asymmetry similar or analogous to that of the crystal or other physical systems. While such molecular or micellar structures are unquestionably present in fibrillae, membranes, skeletal and cuticular structures, shells, and various colloid particles, often constituting local structural patterns, there is at present no evidence that they determine the general order and pattern of the whole organism, and there is considerable evidence that organismic pattern is quite different in character. These molecular and micellar patterns are apparently chiefly characteristic of differentiated protoplasms of certain tissues and of nonliving products of metabolism and of secondary and local significance in development. They occur in all possible orientations with respect to the axes of organisms and even of organs in which they develop. They appear to be effects or expressions of pattern rather than its primary framework.

We may assume, as many biologists have, that the basis of developmental pattern is an inherent property of protoplasm and therefore continuously present and independent of external conditions; but the data of observation and experiment afford little support to this view, for we find no evidence of the existence of such inherent pattern, and we can alter and obliterate patterns and determine new polarities and symmetries experimentally in various ways. If an inherent "intimate structure," such as is often assumed, were the factor determining developmental pattern, we should expect pattern to be less readily alterable by external conditions. Unless we take refuge in so-called "vitalism," which amounts to giving up the problem, we are forced, by the results of experiment, to the conclusion that the axiate individual is the expression of a pattern on a larger scale than molecular or micellar pattern of a protoplasm and that factors external to the individual play a part in determining it. Such a pattern can be determined only by reaction of a protoplasmic system of specific constitution to conditions in its environment, either within the parent body or external. According to this view, molecular, colloidal, chromosomal, and other patterns may be present; but they do not constitute organismic developmental pattern, nor do they autonomously give rise to it.

Organization undoubtedly involves chains of chemical reactions and changes in character of reactions as development progresses, but any conceivable number of chemical reactions cannot give rise to an organismic pattern unless they are in some way definitely ordered in both space and time. The chemical reactions in development are evidently so ordered in a pattern which is spatially of molar order of magnitude and changes in an orderly manner chronologically.

The Roux-Weismann hypothesis that organization results from the separation of hereditary elements by qualitative nuclear divisions during development has been abandoned because neither cytology nor experimental investigation of development support it and because it is difficult to conceive any mechanism or agent, short of a superintelligence, which could fulfil all the requirements of the theory. It is now believed that each nucleus contains all the genes. It is apparently maintained by some that organization is determined by the genes; but if each cell contains all the genes, the cells of a multicellular organism can become different only through action of something external to the genes and determining different gene effects in different cells. Obviously, regions, parts, and organs are localized and become different in the course of development according

to an orderly pattern; that is, a progressive organization occurs. The problem of organization is the problem of the origin and nature of the pattern underlying and determining where, when, and how the differences appear and in what they consist. It involves the physiology and behavior of living protoplasts.

PHYSIOLOGICAL DIFFERENTIALS OR GRADIENTS

Cells commonly have rather sharply defined boundaries, but in the earlier stages of developmental pattern sharply defined boundaries between the various regions or fields of the developing organism are usually absent. Instead of such boundaries, we commonly find evidences of graded differences from one region to another. These differentials, gradations, or gradients appear in various aspects. For example, we find gradations in visible protoplasmic structure and in physical and chemical constitution; in rate of cell division; in cell size; in rate of growth, morphogenesis, and reconstitution; in degree of determination of organs; in respiration; and in reaction to various external factors. Where it has been possible to compare a number of these different gradients in the same individual, a high degree of correspondence as regards region involved and direction has usually been found. The most conspicuous of these gradients and the first to become evident in development are those associated with the polarity and symmetry of the whole organism. In fact, a gradient of some sort is very commonly, if not always, the earliest distinguishable evidence of a physiological axis. Similar gradients also appear in the early developmental stages of various organ systems and organs. Moreover, localized developmental activities very generally show evidence of a graded decrease from a region of highest intensity in a certain or in all directions. It has been possible, in many cases, to alter or obliterate such gradients and to determine new ones experimentally and so to alter the axiate pattern of development correspondingly in definite, controllable manner. The experimental data which will be considered in later chapters show, beyond question, that various sorts of gradients which appear in development are manifestations or expressions of underlying physiological differentials of some sort, which are organismic in order of magnitude; that is, they coincide with the scale of organization of the individual or part concerned. In short, these physiological gradients are characteristic features of axiate order and pattern: differentiation of regions and organs occurs in definite relation to them. Obviously, they are associated with development in some way, either as factors which are operative and determinative or as

incidents or effects of more fundamental factors. Different gradients may differ as regards the activities occurring in them, and the evidence indicates that the presence of a gradient may be as significant for development as its nature. It is evident, then, that consideration of developmental pattern involves the problems of the nature and origin of these gradients and of the parts which they play in development.

THE PROBLEM OF PHYSIOLOGICAL INTEGRATION

The "organism as a whole" is the sum not merely of its parts but also of the relations between them, their actions, and effects on one another. These relations, commonly called "physiological correlation," represent in each particular case a relation of control or dominance, on the one hand, and of being controlled or subordination, on the other. The dominance may range from slight and momentary to complete and permanent. Since these integrating factors are associated with the pattern of organization, they too constitute an orderly spatial and chronological pattern. That physiological dominance exists, even early in development, that it is an essential factor in development, and that in many of the simpler animals it is necessary throughout life for persistence of the individual as a whole have been demonstrated by many lines of experiment.

Present knowledge leads us to conclude that physiological dominance of one region or part over another may be effected in two different ways—by initiation and transmission of energy changes and by production and transport in mass of substances. Dominance of the transmissive type may be regarded as including production and transmission of mechanical, thermal, and electrical changes; but the most important factor is excitation and its transmission. Protoplasm in general is capable of excitation and some degree of transmission of excitatory changes; but the highest development of this type of dominance appears in the nervous system and its receptors, which have become in the higher animals highly differentiated organs of excitation and its transmission. This type of dominance is possible without any pre-existing differentiation from each other of the parts concerned. The region primarily excited is dominant, at least temporarily, over regions to which the excitation is transmitted. The primary excitation establishes the difference between these regions and so determines the dominance. This type of dominance is therefore to be regarded as the primary integrating factor in organismic pattern.

Transportative or chemical dominance presupposes some degree of differentiation of the parts concerned: if differentiation is absent, all parts

will produce the same substances. Dominance of this kind unquestionably occurs widely in organisms but evidently increases in complexity and importance with increasing differentiation of organs, both in individual development and in evolution. The hormone interrelations in the higher vertebrates represent its highest development. Transmissive dominance is a control based primarily on intensity factors; transportative dominance, on specific substances. The one may be regarded as essentially quantitative; the other, as qualitative. Both transmission and transport may be concerned in some types of control, as, for example, in the mediation of nerve impulses by chemical substances. According to current theory, movement of ions occurs in transmission of excitation, but only over short distances; and transmission depends on the electric charges, not on the chemical nature of the ions. The problem of physiological integration of the organism as a whole is the problem of the origin and nature of the factors concerned in dominance and of their effects on subordinate parts. Self-differentiation or independent differentiation of parts, correlative or dependent differentiation, induction, organizers—all raise questions concerning the part which physiological dominance plays in development and the nature of the factors concerned in each case. From another viewpoint, the nature of excitation and transmission, the methods of transport of substances, and the chemical constitution of hormones and other transported substances and of the parts affected are all involved in the problem of physiological integration.

In consequence of the researches of Spemann and his co-workers on amphibian development the terms "inductor" and "organizer" have found wide acceptance among students of developmental physiology. The concepts of inductor and organizer have undergone changes as amphibian experimentation has advanced. The two terms have often been used indiscriminately, but with progress of experiment distinction has become increasingly desirable. At present an "inductor" may be defined as an agent which brings about a definite developmental effect—a determination or differentiation of a particular tissue, for example, or a new axiate pattern. In earlier amphibian experiment the inductors were parts of a developing embryo, either of the same species as that in which the induced effect occurred or of another species. An organizer is an inductor which determines a definite, orderly developmental pattern in another part. The inductors from the region constituting the dorsal lip of the amphibian blastopore are, in general, also organizers, since they determine a new axiate pattern, an organization. As experiment progressed, it was discovered

that more or less differentiation of neural tissue in amphibian embryos could be induced not only by the living tissue of the dorsal lip region but also by various other amphibian tissues of the same or other species, genera, or orders; and not only that, but dead tissues, as well as living, proved to be inductors. With further experiment many living and dead tissues from many different animals, representing most invertebrate and vertebrate groups, tissue extracts, and various synthetic chemical substances, proved to have more or less inducing action. Some of these foreign inductors are apparently organizers: certain plant tissues, for example, have been reported to determine neural plate. Induction is not restricted to amphibian development but occurs in other vertebrate embryos; and in hydroids and planarians small pieces (in planarians chiefly pieces from the region of the cephalic ganglia) may determine new axiate patterns (see pp. 378-87). In the hydroid, *Corymorpha*, a lacerated incision may serve as an organizer.¹ Moreover, photic, electric, thermal, and chemical differentials and gravity can determine new physiological axes and developmental patterns in at least some plant or animal species. These agents are as truly inductors and organizers as living or dead tissues or organic chemicals.² Inductors and organizers are, then, nothing new but are simply cases of physiological dominance established in one way or another and are characteristic of development in general. The character of the dominance remains to be determined in each case. It is probable that it will be found impossible to make any sharp distinction between inductors and organizers, for, as will appear in later chapters, it may be questioned whether the organizer does anything more than determine or play a part in determining a relation of dominance and subordination which becomes the real organizing factor. In view of this possibility, which is supported by experimental evidence, it is perhaps desirable to drop entirely the term "organizer."

It has usually been assumed that chemical substances are the actual inducing agents in vertebrate development. Whether or to what extent this is the case is perhaps still uncertain as regards some inductions. There is, however, considerable evidence which makes it probable that develop-

¹ For earlier experiments showing or indicating dominance and induction in reconstitution see: Browne, 1909; Rand, 1911, 1912; Child, 1911a, c. Many of the experiments of Driesch, Morgan, Child, and others on the hydroid, *Tubularia*, suggested dominance of the hydranth region and of distal over proximal regions. Dominance of the vegetative tip in plants has long been familiar to botanists.

² Experimental determination of axes, inductors, and organizers will be more fully discussed in chaps. xi and xii.

mental induction and axiate organization may be initiated by transmission of excitation in some of the invertebrates. In its more primitive expressions dominance is intimately associated with physiological gradients. The high region of a gradient is primarily the chief dominant region; but at a given gradient level, when isolated from more anterior levels, may, to some degree, dominate lower gradient levels, at least in the polar gradient of hydroids and planarians. Experimental localization of a new dominant region generally results in induction of a gradient or gradient system, with the dominant region as its high end or as its center or otherwise located, according to the form and pre-existing pattern of the material. Transportative dominance in its more highly specific forms is not necessarily associated with a gradient, though there may be an indirect relation if the parts concerned were determined in relation to a gradient.

In many organisms, particularly in the simpler animals, dominance apparently decreases in effectiveness with increase in distance from the dominant region or is limited in range of effectiveness, and this range varies with physiological condition and changes during the course of development. In consequence of this limited range, more or less physiological isolation of parts of the organism which come to lie beyond this range is possible. "Physiological isolation" may be defined as isolation in greater or less degree of a part of an organism from control by a dominant region, without physical discontinuity. Physiological isolation of a part may be brought about in various ways, to be considered in chapter ix. In the simpler organisms the result of physiological isolation is usually alteration of the part of the axiate pattern present or development of a new independent axiate pattern in the isolated part.³

Agamic reproduction in axiate organisms is intimately associated with physiological isolation. In many cases either the degree of isolation is insufficient or the potentialities of the isolated part are too limited to permit development of a complete individual, and a part—for example, a segment—may develop. Local dominances and physiological isolations may be concerned in determining the developmental order of various repetitive parts, such as tentacles. In fact, development apparently involves progressively increasing complexity of relations of dominance and subordination, of both the transmissive and the transportative type. Questions of the origin and nature of physiological dominance in any particular case; of its changes in character during the course of development; of its

³ For earlier discussions of dominance and physiological isolation see Child, 1911a; 1915c, chaps. iv and v; 1924b, chaps. x-xii.



role in determining order, scale of organization, and growth forms of multi-axiate organisms; of its relation to induction; of the significance of physiological isolation in development; and the factors involved in different organisms and under different conditions—these are some of the questions involved in the problem of physiological integration.

THE MATERIAL OF DEVELOPMENTAL PHYSIOLOGY

Comparative investigation of embryonic development in different organisms and groups has given us a broader conception of this form of development than could have been obtained from a single species or group. Comparative study and experimental analysis of different forms of development must provide a basis for a still broader conception and assist us in distinguishing generally essential factors from those concerned with a particular form of development. In all forms of development physiological order, pattern, and integration appear, and evidence is accumulating to show that the essential differences in the different forms are not as great as has often been believed. Some of them present possibilities of experimental analysis which are present to a lesser degree in embryonic development. Sometimes it is even desirable to interpret embryonic development in the light of what we learn from other forms of development.

Embryonic development, development from spores, gemmules, statoblasts, and other special reproductive bodies formed under natural conditions occur in cells or cell masses which have, in each case, a particular past history which is always essentially the same in each species. Some of these reproductive bodies appear to be highly differentiated cells or cell groups, the egg and spermatozoon (the most highly differentiated of all) having undergone greater change from the undifferentiated or embryonic cell than most other cells of the individual. There can be no doubt that the past history of the egg plays some part in determining the course of its development, at least in the earlier stages. In many species there is a considerable degree of regional differentiation in the egg cytoplasm preceding or following fertilization; when present, this may become an important factor in determining the manner in which development takes place, even though the resulting individual may be similar to an individual which develops from a physiologically or physically isolated part of the adult animal or from a bud.

The reproductive cells known as "spores" arise in some unicellular organisms by fragmentation of the individual, but in multicellular organisms they usually originate only under certain physiological conditions and

from certain parts of the body. In some forms the spore is apparently a more highly differentiated cell than other body cells, and in many plants it is formed only in highly differentiated organs; yet it has the potentiality of developing into a new multicellular individual. Multicellular spore-like bodies—sponge gemmules, bryozoan statoblasts, and some ascidian winter buds—have a different past history from that of the egg but can develop into the same sort of individual.

In cases of fission which give rise to new individuals, the reproductive body has previously been merely a part of the parent body. As such it has had a past history of development and more or less differentiation; moreover, it is not always the same part of the parent body which is separated by fission, but the kind of individual which develops from it does not depend on this differentiation. In the flatworms and annelids which undergo fission the course of developmental reorganization may differ according to the body region separated by fission, but similar individuals result. Origin of segments seems to be essentially a repeated fission, primarily mesodermal, with limited development of each. Segments are formed in embryonic development, in the development of zooids and of isolated pieces of segmented animals, but the manner in which they arise differs somewhat in the different forms of development, though the final result may be the same.

Experimental isolation of pieces of the individual by section, with following reconstitutive development, is purely accidental, as far as the original individual is concerned. The different parts have had different past histories and undergo different courses of reorganization in giving rise to similar individuals. Also, they may be of widely different size and develop into individuals of different size; and the animals from which they are taken may be of different age, nutritive condition, etc. Such forms of development provide much more favorable material for various lines of physiological analysis than does embryonic development.

Various multicellular axiate algae separate into individual cells under certain unfavorable conditions, and these cells are capable of developing into new axiate multicellular individuals.⁴ Certain planarian species, if kept above a certain temperature, separate into fragments when they attain a certain size, the size attained and the size of the fragments varying with the character of nutrition (Child, 1913*c*, 1914*d*). At low temperature they become sexually mature instead of fragmenting (Castle, 1928). Certain nemertean species fragment on stimulation. In the planarian frag-

⁴ Tobler, 1902, 1904, 1906; Child, 1917*b*.

ments there is complete degeneration of internal organs during an encysted stage; nevertheless, development of planarians finally occurs. Development of the nemertean fragments is a reconstitution like that following isolation of pieces by section.

In development of new axiate individuals from buds there is much of interest for developmental physiology. The bud makes its appearance as a localized region of cell activation and growth, which in certain plants may begin in a single cell of a leaf or other part of the vegetative body, and in various unicellular organisms, in some portion of a cell. Buds of many multicellular forms, both plant and animal, develop into new axiate multicellular individuals or members of an individuation of higher order, as in multiaxiate plants, hydroids, etc. Moreover, the individual which develops from the bud may be like or unlike the individual from which the bud arises or the individual which develops from the egg. Buds of some forms from different regions of the parent body or from parents in different physiological condition develop into different kinds of individuals; hydranth buds and medusa buds of certain hydroids, and vegetative and flower buds of plants, are examples. Many organs, such as tentacles, appendages, etc., originate as localized budlike regions of growth and differentiation, apparently similar to buds which give rise to complete individuals, but with limited developmental potentialities and often, like the amphibian limb bud (pp. 390-95), deriving a part of their axiate pattern from the parent body. Fusion of eggs, embryos, or other parts may result in the development of single individuals; and transplantation and implantation of parts in embryonic and later stages make possible extensive experimental analysis of determination in parts, of dominance, of induction, and of effects of different organismic environments, different gradient levels, etc., on the course of development.

Since different levels of a physiological gradient differ in susceptibility to many, if not all, external chemical and physical agents which are toxic to living protoplasms, it is possible to alter and control the course of development differentially by subjecting the whole organism to the action of such agents. That the various forms of development provide practically unlimited material for biochemical and biophysical investigation is obvious. Needham's *Chemical Embryology* (1931), which is concerned with the chemistry of only a single form of development, is sufficient evidence on this point. And finally, it is evident that both genetic and environmental factors are concerned in all forms of development. All of them are to be regarded as reactions of protoplasms of specific genetic constitutions to

environmental factors within the parent organism and in the external world. In this reaction certain potentialities of the protoplasm are realized as developmental order and pattern. All forms of development provide material for investigation of the roles of heredity and environment in determining patterns.

In short, embryonic development is not the only, and perhaps in many respects not the most interesting and significant, material for study of various developmental problems. An adequate general theory of development must be based on the less specialized forms of development; it must recognize and distinguish the factors common to different forms of development from those characteristic of only a particular form; also, it must attempt to interpret the more highly specialized embryonic form of development as far as possible in terms of the common factors.

In the following pages a consideration of the physiology of development is undertaken with particular reference to the problems of the origin and nature of developmental pattern. Data of observation and experiment concerning different forms of development, the conclusions which have been or may be drawn from them, and their bearing on the general problem of pattern and particularly on the question whether or to what extent different forms of development may be fundamentally different or fundamentally similar in the essential features of pattern are discussed. Perhaps this survey of developmental physiology on a somewhat broader basis than has usually served—that is, in terms of a comparative analysis of different forms of development with an attempt to discover common factors of pattern—may have some value for the future because it endeavors to show that the patterns of other forms of development are no less, or even more, important than embryonic pattern in relation to the general problem. Perhaps it may also serve as a stimulus or an irritant to further investigation.

CHAPTER II

CERTAIN GENERAL CHARACTERISTICS OF DEVELOPMENTAL PATTERNS

THE various forms of agamic development, reconstitution from experimentally isolated pieces of individuals, and development from the egg have different starting-points and follow more or less different courses. The question of their physiological resemblances and differences is of great interest and importance, since it is a question whether the same organism may arise in fundamentally different ways or whether the different forms of development are physiologically more or less similar in pattern. Observation of different forms of development under natural conditions and of experimentally induced development shows certain features which indicate or suggest that underlying physiological factors may be more or less similar. Certain of these characteristics of patterns which are directly evident are briefly pointed out in the present chapter. Attention has been called to some of them in earlier publications.¹

BUD PATTERNS

In both plants and animals new axes which become new individuals, branches, zooids, organ systems, or organs such as leaves, roots, tentacles,

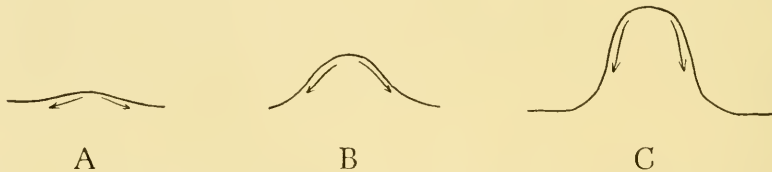
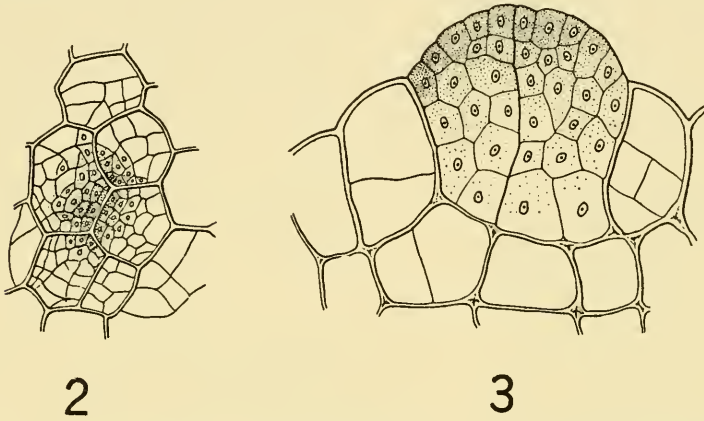


FIG. 1.—Diagrammatic sectional outlines of stages of bud growth; arrows indicate directions of gradients.

appendages of various sorts, develop from what we call “buds.” In its early stages a bud is a locus, usually at or near an external or internal surface of the parent body, in which the conditions which determine its development operate in decreasing degree from a physiological center which may or may not be the geometrical center. These conditions apparently

¹ Child, 1915*c*, pp. 65-87; 1924*b*, pp. 74-100; 1928*d*.

consist primarily in an activation of some sort which decreases in intensity from the center. As the bud develops, the more rapid growth of the physiologically central region brings about elongation, usually more or less vertically to the surface on which the bud appears, and the radial differential becomes longitudinal (Fig. 1). Usually the central region becomes the apical end of the new axis; but in some buds the center may change its position, the axis may become asymmetrical, or the original center may divide into two or more, as in dichotomous branching and various sorts of appendages.



FIGS. 2 AND 3.—Development of an adventitious bud from epidermal cells of a *Begonia* leaf. Fig. 2, surface view; Fig. 3, longitudinal section. The old cellulose membranes are indicated in Fig. 2 by double lines; the membranes of new cells, by single lines. In Fig. 3 the heavier line indicates an old cellulose membrane undergoing resorption (from Regel, 1876).

Adventitious buds which develop from epidermal cells of parts of plants which are physically or more or less physiologically isolated from active vegetative tips provide almost diagrammatic examples of the gradient system and its transformation. Buds of this sort developing from epidermal cells of the leaf blade or petiole in certain species of *Begonia*, after isolation of the leaf, were described and figured by Regel (1876). Before the bud appears, the epidermal cells of the leaf possess cellulose membranes and large vacuoles, have ceased to divide and grow, and under the usual conditions would never divide or grow again. In short, they have all the characteristics of differentiated cells. The locus of a bud may be a single one of these cells, or it may involve several of them. The beginning of bud formation is indicated by disappearance of the differentiated vacuolated condition, accumulation of cytoplasm, and rapid division within the

old cellulose membrane. Figure 2 shows a case in which the locus of bud formation involves most intensely the adjoining parts of four cells. The more deeply shaded cells in the central region are filled with finely granular "embryonic" cytoplasm, and the optical density of this decreases from the center peripherally, as indicated by the decreasing shading. The

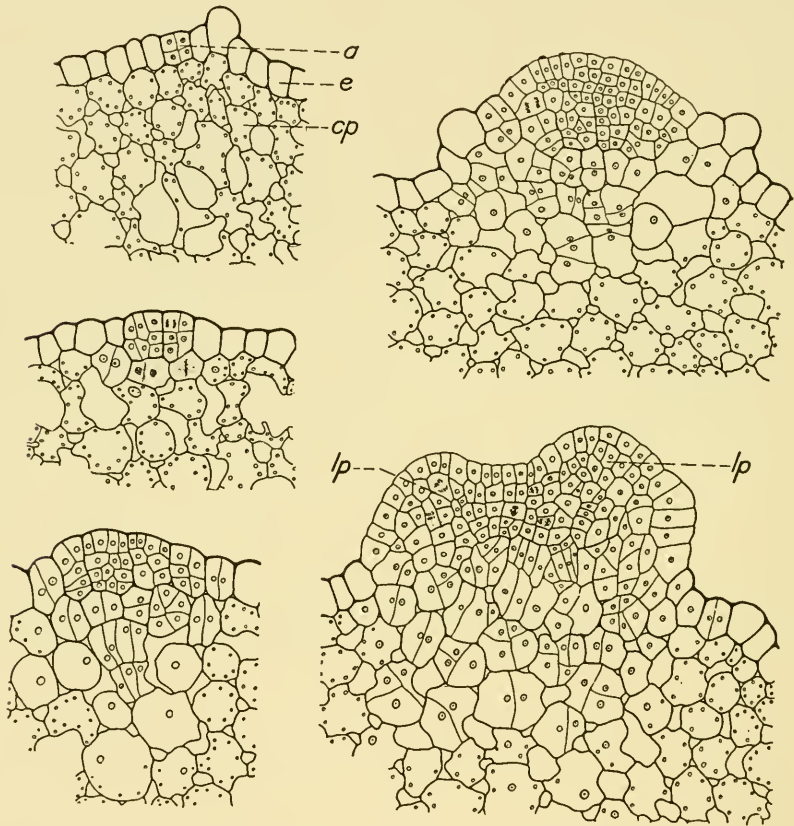


FIG. 4.—Five stages in development of an adventitious bud from an epidermal cell or cells of flax hypocotyl: *a*, four derivatives of single epidermal cell; *e*, epidermis of hypocotyl; *lp*, leaf primordium of adventitious bud (from Crooks, 1933).

peripheral unshaded cells are still more or less vacuolated, although they have undergone division. The figure shows very clearly a radial gradient system in rate of division indicated by cell size and a gradient in the same direction of cytoplasmic character and content of the cells. Evidently the intensity of the activation initiating formation of the bud decreases from the central region peripherally. As development proceeds, the old cellu-

lose membranes are resorbed, and the whole area consists of embryonic growing and dividing cells; but the gradient system persists. Since division and growth are more rapid in the center of the locus than peripherally, the developing bud grows out from the leaf surface, and the radial gradient system becomes an apicobasal axial gradient system. Figure 3 shows a vertical section of a bud after outgrowth has begun. It is evident that the gradient in cell size and cytoplasmic content extends from the free surface proximally as well as from the center peripherally. In the stages shown in Figures 2 and 3 the bud represents the apical region of the new axis. With further elongation the free end becomes the vegetative tip, leaf buds appear in definite relation to it, and differentiation of vascular bundles begins at a certain distance from it.

In the seedlings of flax (*Linum usitatissimum* L.) development of adventitious buds can be induced from epidermal cells of the hypocotyl by removal of all buds distal to this region. Five stages in development of these buds are shown in Figure 4. In the earliest stage (upper left) only a single cell has begun to divide; the later stages figured show a general radial and basipetal gradation of increasing cell size, indicating decrease in rate of division from the region of primary activation. In most cases among the seed plants adventitious buds do not arise from epidermal cells but from tissues below the surface, but the visible pattern does not differ fundamentally from that described here. Adventitious buds may arise in callus tissue which develops on wounded surfaces in many woody plants. Here, also, bud pattern is essentially similar to that of the epidermal bud. Roots also develop from buds which originate beneath the surface of the plant body. As in other buds, the apical region develops first, and other parts arise from it as it elongates.

So far as gradient pattern is concerned, early stages of animal buds are very similar to those of plants. Figure 5, an early stage of amphibian limb buds, is of interest in comparison with the figures of plant buds. The decrease in number of nuclei per unit area from the mesodermal region in contact with the ectoderm suggests an activity gradient of some sort. In the relation between the primary gradient pattern of the bud and morphogenesis the differences between plant and animal bud development are much like those between embryonic plant and animal development. In the plant bud the apical region usually remains for a time or indefinitely an embryonic growing region, and morphogenesis begins at a certain distance from it. As new material is added to the axis from the tip, each level begins morphogenesis when the critical distance between it and the tip is

attained. In the animal bud morphogenesis usually occurs first at the apex and progresses basipetally, and the region of most rapid growth may also shift basipetally and perhaps becomes basal or posterior at some later stage. In certain syllid annelids the posterior segment-forming region buds, giving rise to new regions like itself, which become the apical regions of the bud axes (except for the anal segment) but actually represent posterior body regions and produce new segments anteriorly, the first formed becoming the head (H. P. Johnson, 1901, 1902). In these buds the new segment-forming regions remain embryonic for a time and give rise successively to new segments; they and the posterior segment-forming regions



FIG. 5.—Early stage in development of amphibian limb bud in section (from Filatow, 1933).

of annelids in general, as persistent growing regions, are somewhat similar to the vegetative tips of plant axes.

In its most primitive form bud pattern becomes evident as primarily a radial gradient system; but many buds, both plant and animal, show earlier or later a bilaterality or an asymmetry, usually definitely related to the axes of the parent body. The bilaterality of many leaves and flowers, the dorsiventrality of lateral branches of many conifers and other plants, and the anteroposterior and dorsiventral asymmetry of the amphibian limb indicate that the axiate pattern of the region from which the bud arises persists in, or is impressed on, the bud.

In general, budlike developmental loci, whether plant or animal, whether developing into new individuals or into organ systems or organs, show very similar gradient patterns in early stages. Unquestionably, the nature

of the physiological differential from center to periphery differs in different kinds of buds, but the relation of the differential to center and to periphery is very similar; and in the adventitious bud of the plant or the bud which becomes a new zooid in the hydroid or a new appendage in the amphibian—the pattern—originally more or less radial, becomes longitudinal in consequence of differential growth.

Buds which develop into complete individuals must finally undergo complete separation from the parent body. Usually the free end of the bud axis becomes apical or anterior; the attached end, basal or posterior. The fission which separates bud individual from parent occurs when the attached region has attained a certain stage of development. A familiar example is the hydra bud. In the buds of the segment-forming region of certain syllids mentioned above, the head develops from the attached end and its developmental stage determines separation, but the primary bud pattern is apparently similar to that of other buds. So-called “buds” of some forms—for example, the “winter buds” of some ascidian species—are really fission pieces or cell aggregates rather than buds and separate from the parent body before any development occurs. It seems preferable to restrict the term “bud” to that form of development in which a new axis originates in a local activation and differential growth.

In some axes developing from buds or budlike outgrowths the region of most active growth remains at the base instead of becoming apical. The hairs of certain algae, the long bladelike leaves of various plants, and probably some animal appendages are examples. These axes represent progressive differentiation from base to tip during growth or development; but to what extent this type of axis is an integrated whole, not merely a differentiation gradient of a certain kind, is not known. This kind of bud axis is much less common than that with the high end of the gradient at the tip. Some of these “inverse” bud axes—perhaps all of them—are apparently similar to the usual type of bud in their early stages, and in some the region of most active cell formation and growth may shift from base to tip in the course of development. Most of the bud axes mentioned in the following pages are of the usual type, with the region of most intense activation becoming the tip, at least primarily. The inverse bud axes usually, if not always, give rise to differentiated parts, not to entire individuals.

Certain questions concerning bud pattern, conditions which determine the origin of a bud, and other lines of evidence concerning its pattern, organism bud, and organ buds are taken up in later chapters.

MULTIAXIATE GROWTH FORMS

The branching or multiaxiate plant, whether alga or tree, originates as a series of buds from the primary axis. Each branch of the common type of multiaxiate plant consists, at least during its development, of a vegetative tip, which is the first part formed, and other axial levels formed by the tip. Protoplasmic growth decreases basipetally in the tip, except as new bud loci are activated in certain spatial relations to the tip; and other forms of growth, such as increase of cell size by increase in size of vacuole, usually also decrease basipetally from a certain level below the tip where they begin. Cells in certain regions of these lower levels, however, may still continue division and protoplasmic growth and bring about increase in transverse diameter along the axis. It is evident that the growth form of the whole multiaxiate plant must be determined by relations between the different axes. The tapering growth form of the fir and many other conifers, of various other trees and herbaceous plants, and of many algae with branches radially arranged or in a single plane must result from relations of some sort between the main axis and lateral branches. Moreover, the variations in this growth form, that is, from a short cone with large base to a long cone with small base, and the almost cylindrical, ovoid, or nearly spherical forms of some other trees can result only from orderly, graded differences in growth of the different axes, differing in different species. Many multiaxiate plants—many trees, for example—show, when young, a more or less conical form with marked difference in growth and form of the main axis and lateral branches; but later these differences between main and lateral axes may decrease, and all may become more or less completely equivalent and a form with spreading crown of more or less similar branches results. The quantitative study of growth of lemon shoots by Reed (1928) shows differences in growth of apical and lateral shoots of definite and orderly character. Plants with a multiaxiate inflorescence usually show a gradient, either acropetal or basipetal, in development of flowers. In the cotton plant, for example, blooming begins at the bases of the lowest fruiting branches and progresses acropetally along each branch and along the main axis (McClelland, 1916). Many other examples of both acropetal and basipetal progress of blooming might be given. Which of the two orders occurs apparently depends on the condition of the tip at the time of flowering. If it is still growing, the order of flowering is, in general, acropetal; if early transformation of the tips into flower axes occurs, the order is basipetal.

The growth form of the multiaxiate ciliate protozoan *Zoothamnium* is

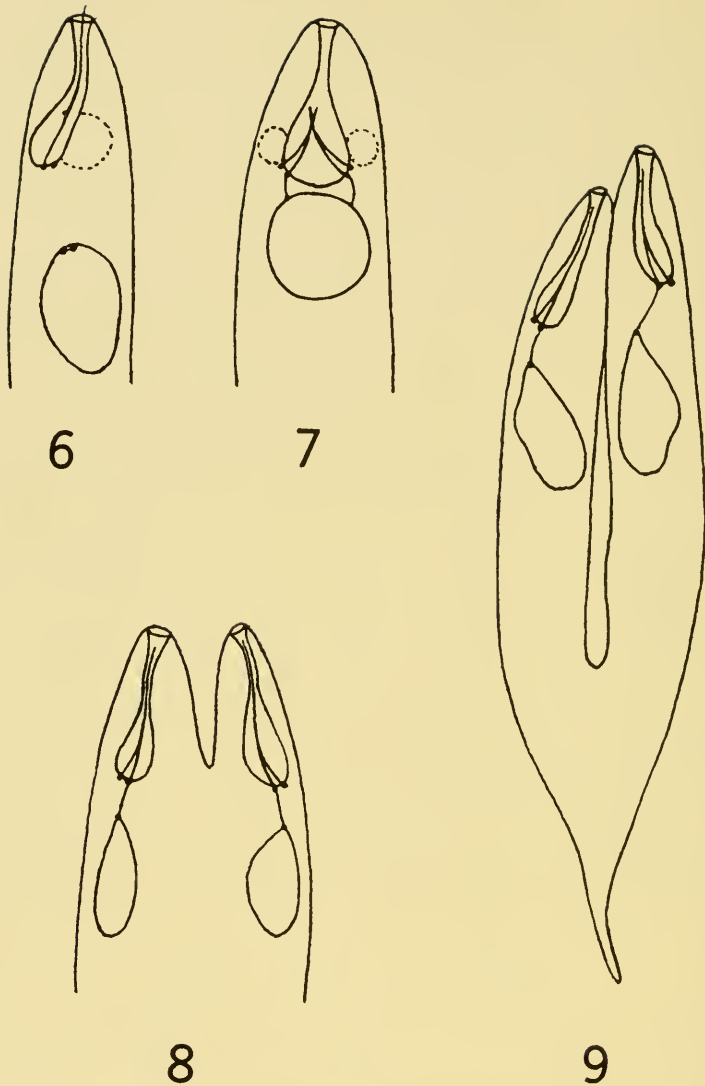
much like that of a conifer with single main axis and lateral branches, except that the branches lie in one plane. Fauré-Fremiet (1930) has shown that in *Z. alternans* the colony, as a whole, shows a certain degree of individuality in the distribution of growth and multiplication of its cells in a graded order. The zooid axes of certain other branching ciliates—*Carchesium*, *Epistylis*, etc.—are apparently equivalent, and the growth form is similar to that of a plant with a spreading crown of equivalent branches.

The branching hydroids and bryozoa show growth forms very similar to those of multiaxiate plants and apparently resulting from similar relations between different axes. In certain species of *Pennaria* and *Obelia*, for example, the growth form, when not altered by injury, crowding, or other conditions, is in general conical like that of conifers. In many other hydroids the branches are more or less equivalent as regards growth; and the spreading, less regular growth form results. An acropetal gradient in time of appearance of medusa buds, whether on the hydranth bodies or in special zooids, occurs in many species of branching hydroids. Apparently, lower levels of the multiaxiate whole arrive at the physiological condition which determines formation of medusa buds earlier than higher levels. Buds which develop into new individuals arise in definite graded orders on stolons in certain medusae, siphonophores, and ascidians. This order results in gradients of size, of stage of development of the buds, and often of arrangement of buds; in some forms these gradients are repeated in orderly sequence, indicating periodic or cyclical changes in condition.² In general, the character of multiaxiate growth forms is determined by the spatial and chronological order of development of the buds which constitute the axes and by the rates of growth of the different axes. All these indicate the existence of graded differentials of some sort.

PATTERN IN RELATION TO FISSION

Fission in many of the simpler unicellular organisms is apparently nothing or little more than cell division; but in many forms with definite axiate pattern which reproduce by fission, a definite developmental pattern becomes evident in the reconstitution of the parts resulting from fission. Brief mention of a few cases will serve to illustrate the point. Many of the flagellate protozoa divide longitudinally, with cytoplasmic division beginning at the anterior end and progressing posteriorly. Figures 6-9

² For interesting examples of these orders see Chun, 1896; Braem, 1908, for budding of medusae; W. K. Brooks, 1893; M. Johnson, 1910; Ritter, 1911, for bud orders in salpid ascidians.



FIGS. 6-9.—Four stages of cell division in *Euglena spirogyra*; chromosomes and nuclear division not shown. Fig. 6, intranuclear body which forms the blepharoplasts has moved to anterior end of nucleus and divided. Fig. 7, two blepharoplasts, resulting from a second division of the intranuclear bodies, have reached the base of the reservoir, where the flagellum arises; new axial fibers have developed from them and united with the old, and the flagellum has divided. Figs. 8 and 9, later stages, showing progress of division of cell body from anterior end (after Ratcliffe, 1927).

show four stages in the cell division of *Euglena*, as described by Ratcliffe (1927). The nucleus approaches the anterior end before division, and the behavior of the blepharoplasts and the progress of cytoplasmic division from the anterior end posteriorly indicate that physiological conditions in the anterior region of the cell differ in some way from those in more posterior parts. In certain choanoflagellates division progresses posteriorly in the cell body and anteriorly in the collar. Division in the cystoflagellate *Noctiluca* progresses over the body from the mouth region, which is undoubtedly to be regarded as apical. Division of certain trichomonad flagellates progresses from the anterior end posteriorly (Kofoed and Swezy, 1915). In *Noctiluca* and the trichomonads the nucleus is situated in the anterior end, but in others which show the same course of the division furrow from anterior to posterior this is not the case. In the two species of *Spirotrichonympha* recently described by Cleveland (1938) the reconstitution of the highly differentiated morphological structure in connection with fission progresses basipetally from the apical end of the new individual, which in one species is half the apical end of the original animal and in the other is a new apical region at the original posterior end. Longitudinal division also occurs in the stalked ciliate protozoa such as *Vorticella*, *Carchesium*, *Zoothamnium*, etc., the division furrow appearing first at the distal end of the zooid and progressing proximally. It is well known that in *Stentor* and various other forms the peristome region develops first in fission; development of the new gullet in fission of the heterotrichous ciliate *Bursaria* progresses from the anterior end (Lund, 1917).

Indications of a longitudinal differential also appear in free-swimming ciliates which divide transversely, though these are usually less definite than in flagellates and sessile ciliates. When the temperature of a *Paramecium* culture is raised from 18°–22° C. to 26°–30° C., the anterior member of a pair resulting from fission undergoes the following division earlier than the posterior member; but when the temperature is lowered from 18°–22° C. to 13°–17° C., the posterior member divides first. Even in the third generation at high and low temperatures some indications of this difference persist (W. Petersen, 1927). The author interprets these results as due to a difference in physiological condition in anterior and posterior regions which results in a differential susceptibility to high and low temperatures, the high temperature accelerating, the low temperature depressing, the anterior more than the posterior member. De Garis (1928) followed division rates in five lines of *Paramecium* with selection for anterior and posterior origin. In three lines the anterior selections showed a

higher mean fission rate; in one rates were equal, and in another the rate was higher in posterior selections. These data, particularly those of Petersen, suggest an anteroposterior differential in physiological condition but indicate that, although the differences in the products of division may be inherited, they do not necessarily persist indefinitely.

A more or less complete replacement of peristome and its membranelles and of the cirri occurs in both members in fission of hypotrichous ciliates. The new cirri appear in certain areas or fields before the old cirri disappear. In *Euplores* the primordium of the peristome is the first indication of reorganization of the posterior product of fission, the primordia of the cirri appearing somewhat later (Wallengren, 1901). This suggests a gradient in the developmental field of the posterior member. From certain of his figures (*H* and *J*) of *Stylonychia* it appears that the new cirri of the anterior member are slightly more advanced in development than those of the posterior member. If this is true, it suggests an anteroposterior gradient in the parent individual.

The gradient of the parent body is clearly evident in the successive fissions constituting strobilation of the scyphistoma of *Aurelia* and other discomedusae. The longitudinal fissions which occur occasionally in hydra and actinians, either as the result of injury (Roudabush, 1934) or other conditions, begin apically and progress basipetally. Fission also occurs in many flatworms, nemerteans, and annelids. In some cases it is essentially fragmentation without definite fission zones, as in some nemerteans and annelids. In others fission occurs at definite body-levels, and visible reconstitution may precede or follow separation. Reconstitution of a whole from the part in connection with fission in these forms is essentially similar to reconstitution following experimental isolation of pieces by section, which is discussed below. Usually a part of the parent pattern persists in the fission piece and becomes the starting-point for orderly reconstitution. Fragmentation of nemerteans, *Lumbriculus*, and other forms apparently results from strong muscular contraction without predetermination of body-level. Separation at a definite body-level precedes morphological development in *Dugesia* (= *Euplanaria*) *dorotocephala* and some other planarians; but the new developmental pattern is physiologically distinguishable (pp. 108-17), and separation occurs when its physiological isolation becomes sufficient to permit an independent motor reaction. In many forms development precedes separation so far that chains consisting of several zooids may result, separation occurring when head and posterior end of adjoining zooids attain a certain degree of development. Figure 10

shows four stages in development of a chain in the rhabdocoel, *Stenostomum*. The second and following fissions occur in order from anterior to posterior zooid of the chain; that is, increase in length of a zooid sufficient to permit determination of a new head region, which can occur only at a certain distance from the head of the zooid, is most rapid in the anterior

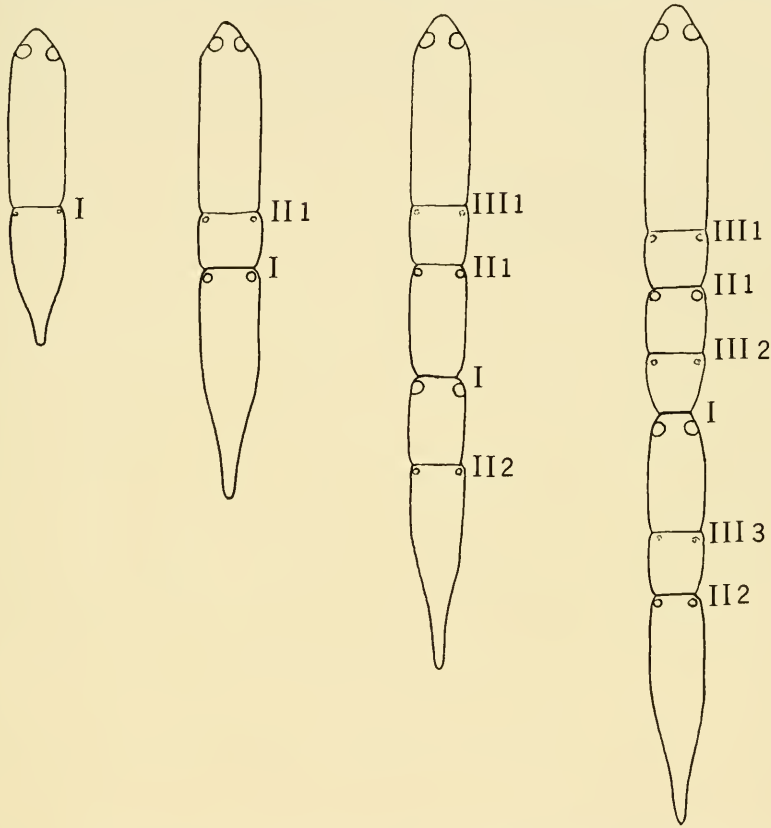


FIG. 10.—Four stages in development of zooid chain of *Stenostomum grande*; fission zones of first, second, and third order are indicated by Roman numerals; the order of their appearance in second and third series, by Arabic numerals.

zooid and less rapid from zooid to zooid posteriorly, as the figure indicates. Consequently, the chain shows a gradient pattern as regards order of appearance of new zooids, and each fission plane apparently results from determination of a new head region in consequence of a certain degree of physiological isolation of the body-level concerned from the dominance of the head region of that zooid. Among microdrilous oligochetes similar pat-

terns occur, but the chains usually consist of fewer zooids because growth in length and physiological isolation of the posterior regions of existing zooids are relatively less rapid than development of zooids. In some polychete annelids chains of zooids occur, but the posterior members usually separate successively before they undergo fission. Fissions provide examples of reconstitution occurring under natural conditions, in consequence either of physiological isolation or of physical isolation. The polarity and ventrodorsality of the new individual are usually in the same direction as in the parent: the new head develops at the most anterior region of the part, which becomes the zooid, and its ventrodorsal pattern is apparently determined by the parent ventrodorsality. Except as regards effects of physiological isolation of parts in initiating development, the natural fissions give us little information concerning developmental pattern that is not obtainable from the reconstitutions resulting from experimental section, and they have the disadvantage for experimental analysis that they occur only under certain physiological conditions, although these conditions can be induced experimentally in some forms, and are usually limited to certain regions of the body. The wider range of possibilities of analysis in the reconstitutions following experimental section will become evident in the following section.

POSTEMBRYONIC RECONSTITUTIONS

Reconstitucional development may be defined as the alteration of pattern and course of development in a part of a pre-existing individual following its physical or physiological isolation from its normal organismic environment. In other words, it is the realization, in the isolated part, of other developmental potentialities than those which have been, or would be, more or less fully realized without isolation. This form of development has been variously called "regeneration," "restitution," "reparation," "reproduction," and, in general, "form regulation." For Driesch and many others it has involved the assumption that pattern and development of the isolated part are so altered that it approaches in some degree or becomes a whole, a normal individual; that is, the isolated part gives rise to more of the individual than if it had remained an integrated part of the original whole. This conception of reconstitution is implied in the terms "restitution," "reparation," "regeneration," and "form regulation." While it is, of course, true that very often an isolated part does become more nearly a whole, it is also true that very often it does not. Its pattern may be so altered that it gives rise to some other part or parts of

the individual than those which it formed or would form without isolation; but whether these represent more, or possibly in some cases less, of the whole depends entirely on what we mean by "more" or "less." For example, when a short piece of a *Tubularia* or *Corymorpha* stem develops into only the extreme oral end of a hydranth or to two opposed oral ends (pp. 334, 346), has it become more nearly a whole than it was originally? Unquestionably, its developmental pattern has undergone alteration; but what has occurred can scarcely be called a restitution, a reparation, a form regulation, or even a regeneration in any strict sense. It is, however, a reconstitution, but one which makes long-continued existence impossible. It is perhaps even pertinent to raise the question whether continued differentiation of an isolated part in the same way as without isolation may not, at least in some cases, involve some reconstitution. The fact that the isolated part is capable of more or less independent or self-differentiation does not prove that it differentiated independently when not isolated. In view of the experimental data the term "reconstitution" seems preferable to any of the others used for this form of development, because it involves no implications of approach to wholeness, regulation, or replacement of missing parts. It implies nothing beyond alteration of developmental pattern in the isolated part. As already noted, physiological or physical isolation and reconstitution of parts occur in the various forms of budding and fission. Either a new axiate pattern develops in a former part of an individual, as in buds, or the partial pattern present undergoes reconstitution, usually into the pattern of a whole. It is perhaps worth pointing out that even embryonic development may be regarded as reconstitucional in character. The egg and the spermatozoon are morphologically, and apparently also physiologically, highly differentiated and specialized cells, and in embryonic development the differentiation and pattern undergo great alterations. For parthenogenetic eggs isolation from the earlier organismic environment is sufficient to initiate development, but in other eggs activation by the spermatozoon or by chemical agents is also necessary. In any case the egg does not develop so long as it remains as a part of the parent body in the ovarian environment. It is difficult to understand how anyone can observe the changes which egg and spermatozoon undergo in fertilization and development and doubt or deny the occurrence of dedifferentiation. If the gametes are, in any sense, parts of the parent body during their development as gametes, embryonic development is a more extreme reconstitution than any other form of development.

The chief purpose of the present section, however, is to call attention to some of the graded differences in reconstitucional patterns of experimentally isolated parts of more or less mature individuals and particularly to their relations to body-level of origin of the part. The reconstitutions of mature organisms, particularly of those with elongated polar axes, usually show such differences more clearly than those of isolated parts of eggs and early embryos. Experimental analysis of differentials along an axis in the egg and in early embryonic stages, by isolation of parts, is to some extent limited by the presence of the whole axiate pattern in one or a few cells and often also by the regional cytoplasmic differentiations which occur in many eggs. These apparently represent developmental expressions of pattern in relation to ovarian environment, maturation, and fertilization, rather than the fundamental pattern itself. For these reasons consideration of egg and embryonic reconstitutions is postponed to a later chapter, following presentation of other lines of experimental analysis of patterns of various forms of development.

For convenience three types of reconstitution may be distinguished: substitution, redifferentiation or reorganization, and regeneration. Substitution is reconstitution in multiaxiate forms by substitution of another axis for one removed. Substitution occurs very commonly in multiaxiate plants in consequence of isolation from a dominant region. Removal of a dominant axis or of its dominant region results in reconstitution of another, or of more than one, axis (previously subordinate) into dominant axes; or in initiation and development of one or more new axes—adventitious axes—from cells which were previously parts of an axiate pattern already present. In certain conifers, for example, removal of the dominant region of the main axis, the vegetative tip, is followed by a turning-upward of one or more of the uppermost lateral branches and its transformation from a bilateral to a radial pattern of branching. Bud primordia inhibited by a dominant region of another axis may be activated and may develop following removal or inhibition of that region; or, if no bud primordia are present, adventitious buds may develop following isolation from a dominant region. Substitution may occur in multiaxiate animals. A hydra bud may remain attached and become the apical region if the parent body distal to the bud-level is removed.³

Redifferentiation or reorganization is reconstitution of an isolated part into something else without outgrowth of new tissue from surfaces of section. A piece of *Tubularia* stem, for example, may undergo reconstitution

³ Weimer, 1928; Rulon and Child, 1937*a*.

into a hydranth or the distal part of a hydranth by reorganization or redifferentiation without any "replacement of missing parts" by outgrowth of new tissue.

Regeneration is reconstitution by outgrowth of new tissue from the cut surface and its differentiation into a part of an individual, often, but by no means always, more or less similar to the part removed. Regeneration, as defined, occurs widely in animals but is not common among plants, though some algae and fungi regenerate and parts of vegetative tips removed may be regenerated in some of the higher plants.

Redifferentiation and regeneration, as here defined, are probably not very different physiologically. Reconstitution in stem pieces of the hydroids *Tubularia* and *Corymorpha* is wholly redifferentiation; there is no replacement of parts removed, but transformation of the piece. In most planarians both regeneration and redifferentiation occur in reconstitution, and the proportion of each differs in pieces from different body-levels of the same individual and can also be altered experimentally. In general, redifferentiation occurs to a greater extent in the less, regeneration in the more, stably differentiated forms, even within the same phylum or class. In *Dugesia* (= *Euplanaria*), for example, considerable redifferentiation occurs; in *Procotyla*, another triclad of different family, almost none. Reconstitution in later developmental stages of arthropods and vertebrates is limited to regeneration of certain parts, such as appendages and various tissues.

The experimental reconstitutions resulting from isolation of parts by section provide material for experimental analysis which cannot be obtained in other ways. In various animals they can be initiated at will; and the region of the body, size, and form of the isolated part can be varied and controlled within wide limits, the effects of nutritive condition, physiological age, and other factors can be investigated, and much concerning physiological dominance can be learned.

PLANT RECONSTITUTIONS

A few examples of reconstitution in plants are briefly described. Reconstitution in certain algae shows graded differences along an axis which are very similar to those in hydroids. The thallus of the alga *Acetabularia mediterranea* consists of a long stem from which whorls of hairs develop, an umbrella-like apical region, and rhizoids at the basal end, all formed from a single cell with the nucleus in the rhizoid region. Nucleated pieces reconstitute completely, and even nonnucleated pieces of the stem are

capable of considerable reconstitution. The original polarity is usually retained, but short pieces may give rise to bipolar forms. Distal nonnucleated pieces reconstitute apical parts; proximal pieces, rhizoids more readily or more completely (Hämmerling, 1934*a, b, c*; 1936). To account for the observed results Hämmerling postulates two opposed gradients of different formative substances produced by the nucleus and accumulating in concentrations decreasing from each end of the axis. The differences in reconstitution in pieces from different body-levels are not very different from those in certain hydroids. For the hydroids, however, the hypothesis of two opposed gradients of different formative substances advanced by Morgan was later abandoned as inadequate. Since these substances are assumed to accumulate toward opposite poles of the axis, their distribution must be determined by a polarity of some sort to which they react. If this be granted, the possibility that that polarity, rather than the hypothetical formative substances, is the essential factor in determining the differences in reconstitution at different body-levels may perhaps be admitted. Undoubtedly, the nucleus is essential for the continued life and activity of the cytoplasm, but whether it is responsible for the axiate pattern appears open to question. It does not appear that the nucleus has any mechanism for determining the distribution of the two formative substances in two opposed gradients, even if the hypothesis of the substances and their distribution be accepted. But whatever the interpretation, the experiments show the existence in this single cell body of a gradient pattern very similar, as regards its effect on reconstitution, to that in hydroids and various other animals.

The alga *Caulerpa prolifera*, a single multinucleate cell with differentiation into rhizomes, rhizoids, and leaflike parts, also exhibits interesting evidences of a gradient pattern in reconstitution.⁴ In certain fungi presence of a gradient pattern and dominance of the apical region are indicated by the data of reconstitution.

An interesting example of substitution by formation of adventitious axes appears in the liverwort *Marchantia*. The thallus pattern resembles that of a bilateral animal in that it consists of a longitudinal and dorsiventral axis with the dominant region at one pole (Fig. 11, *A*). The notched end may be regarded as anterior, for the apical cell, which constitutes the growing tip and gives rise to other cells, lies in the notch, and this is the dominant pole. The apical cell at times undergoes dichotomous division, giving rise to two apical cells, each of which becomes the anterior pole of

⁴ Zimmermann, 1929, and references to earlier papers given by him.

a new thallus. Isolated pieces of the thallus reconstitute with interesting evidences of gradient pattern. Any cell of the thallus is capable of reconstituting a new thallus, but in multicellular pieces preferential localization of reconstitution occurs (Vöchting, 1885). When an anterior region is removed by transverse section at any level, a new apical cell and a new thallus develop from the ventral side of the midrib just posterior to the cut surface (Fig. 11, *B*). In general, in pieces containing any part of the midrib the new thallus develops from its anterior ventral region (Fig. 11, *C*, *D*). In pieces entirely lateral to the midrib with transverse anterior cut

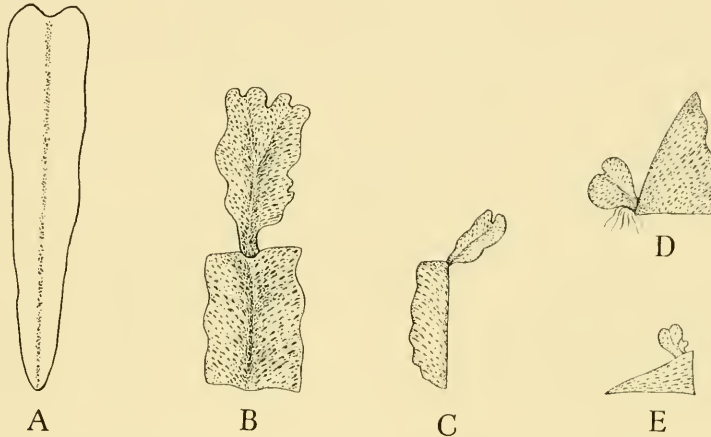


FIG. 11, *A-E*. Reconstitution in the liverwort *Marchantia*. *A*, unbranched thallus with midrib indicated; *B*, reconstitution from ventral side of midrib after transverse section; *C*, *D*, reconstitution from half-midrib in pieces of half-thallus width; *E*, reconstitution from most anterior level of piece without midrib and with anterior oblique cut surface (from Vöchting, 1885).

end, the new thallus develops from the most nearly median part of the anterior region; but in pieces lateral to the midrib with oblique anterior section, development is from the most anterior region of the piece, which is at the lateral border (Fig. 11, *E*). These and various other experiments indicate the existence in the thallus of a longitudinal and mediolateral gradient pattern of some sort. Any level of either can be experimentally determined as the locus of development by altering the shape of the piece and the region included in it. Planarian pieces of different shape and including different regions give results which are similar in certain respects as regards the gradient pattern which they indicate (pp. 52, 364-65).

In multiaxiate seed plants the chief growing tip may completely in-

hibit or greatly retard development beyond a very early stage of bud primordia below it or may determine their development as lateral branches. When this tip is removed or inhibited, the usual result is that the uppermost buds develop, or one or more of the uppermost branches reconstitute a main stem and development or transformation of axes below is inhibited. On removal of the reconstituted axis or axes the next uppermost buds or branches react first, and so on. Even in the potato tuber, which is a modified subterranean stem, this relation appears to some extent (Appleman, 1918, 1924). In certain other plants only a difference in rate or time of development of buds along the stem results from removal of the tip, the uppermost buds developing first, and others in basipetal order. The relation of development of lateral roots to a main root tip is very similar.

Adventitious buds which develop from differentiated cells in some plants after removal of all growing tips and preformed buds may also show preferential localization or more rapid development at more apical levels. When they develop on pieces of roots, as in the dandelion (Nemec, 1908), they tend to be localized at the basal end of the piece, that is, on the end toward the stem; but in very short pieces they may appear anywhere without preferential localization. The data indicate a gradient pattern more or less effective in determining localization of the buds in longer pieces but ineffective in very short pieces because in them there is so little regional difference. Rhizoids and roots usually show an opposite localization differential from shoot buds, that is, they tend to appear basally on isolated stem pieces; they can, however, be determined at any level.

The art of pruning, trimming, and "forcing" development of certain axes by removal of others in plants depends on reconstitution by substitution and on presence of a gradient pattern of some kind along the axes concerned.

RECONSTITUTION IN ANIMALS IN RELATION TO BODY-LEVEL

CILIATE PROTOZOA

Some indications of a longitudinal gradient appear in connection with fission in ciliates (p. 24); and, although experiments on reconstitution have usually been chiefly concerned with other problems, they add some evidence. Reconstitution of the gullet of *Bursaria* after section progresses from the anterior end, as in fission (Lund, 1917). The multiple monsters which sometimes result from incisions in the cell body of *Paramecium* may show a considerable number of partial axes, each of which represents at

first an anterior region, the posterior part of the body being undeveloped (Fig. 12). Development of some of these may continue posteriorly until a complete individual separates from the mass (Calkins, 1911). More recently Sonneborn (1932) has described monsters of the ciliate *Colpidium campylum* which appear very similar to the *Paramecium* monsters but result from culture with a strain of *Micrococcus* as food. *Stylonychia mytilus* has a micronucleus and meganucleus in the anterior half of the body and

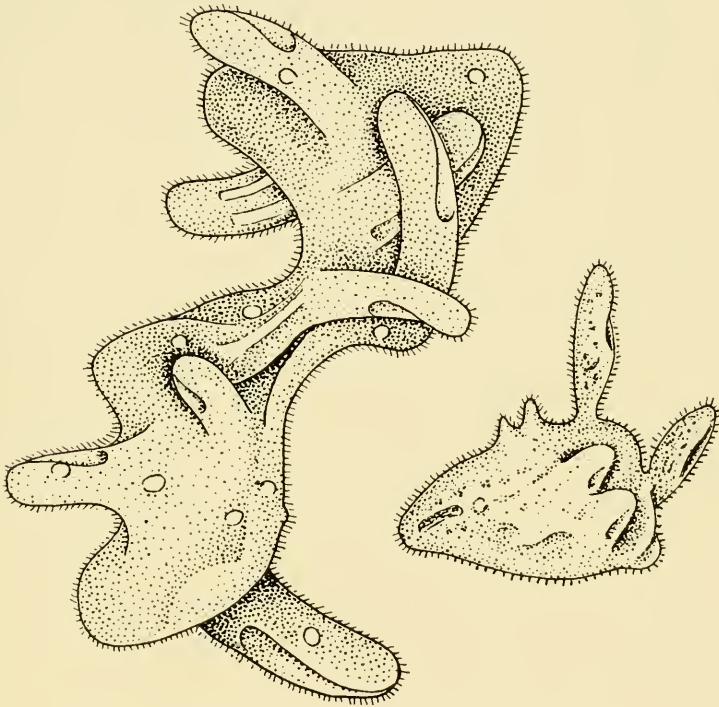


FIG. 12.—Multiple *Paramecium* monsters (from Calkins, 1911)

another similar pair in the posterior half. In fission, the two fields from which the cirri develop are localized near each pair of nuclei. When reconstitution occurs after any injury which leaves the two pairs of nuclei intact, the field from which the new cirri develop appears in relation to the anterior pair of nuclei. Only when these nuclei are removed does a cirrus field develop in relation to the posterior nuclei. The field of the marginal cirri always develops in the anterior half of the body, and development progresses from anterior to posterior (Dembowska, 1925).

COELENTERATE RECONSTITUTIONS

Decrease in rate of tentacle development and in number of tentacles appearing in a given time from distal to proximal levels of section has been observed in pieces of hydra (*Pelmatohydra oligactis*).⁵ Reconstitution occurs at all levels of the stem or hydrocaulus of the hydroid *Tubularia*, which in some species may attain a length of 10 cm. or more without



FIG. 13.—Hydranth reconstitution in *Tubularia*; a long hydranth primordium at oral end of a distal stem-level and a short aboral primordium from a proximal level.

branching. The hydranth primordium develops within the perisarc adjoining a cut end by redifferentiation of the coenosarc, the tentacles arising as longitudinal ridges and separating from the hydranth body progressively from tip to base (Fig. 13). Emergence of the hydranth is brought about later by elongation of the coenosarc proximal to it. Evidently hydranth development progresses from the tip of the piece proximally. Further evidence of this course of development is given by the reconstitution of very short pieces into apical parts of hydranths. In these as much of the hydranth is formed basipetally as length of piece and scale of organization permit (pp. 344-49). Pieces a centimeter or more in length usually give rise to a hydranth at each end (oral and aboral hydranth); but, in general, results of reconstitution vary with length of piece, body-level, and condition of animal.⁶

Rate of hydranth development, as measured by time from section to emergence from the perisarc, is higher at the distal than at the proximal end of the piece, except when very short; the rates at the two ends may then be equal, because the gradient is practically absent. Sometimes, also, the rates may be almost equal at the two ends of extremely long stem pieces, apparently because the proximal end has become physiologically isolated from the dominant hydranth and has undergone change in physiological condition as a result. Some species of *Tubularia* form buds and new axes under these conditions, that is, when proximal regions come to lie beyond the range of dominance of the apical hydranth or when its domi-

⁵ Peebles, 1897; Koelitz, 1910; Weimer, 1928; Rulon and Child, 1937.

⁶ For experimental data see Driesch, 1897, 1899b; Morgan, 1901b, 1902a, 1903a, 1905, 1906b, 1908; Morgan and Stevens, 1904; Child, 1907a-f.

nance decreases. In pieces of equal length from different stem-levels rate of hydranth development decreases at both ends of the piece with increasing distance of the level of reconstitution from the oral end of the stem, but in extreme proximal regions the differences may be slight or absent.

Although all earlier investigators who concerned themselves with the question of rate at different levels of the *Tubularia* stem were essentially agreed as to the presence of these differences, Garcia-Banus (1918) presented experimental data to show that they do not exist. Hyman (1920b), however, showed conclusively that they are present in animals in good condition and pointed out that the rates recorded by Garcia-Banus were so much lower than others had found them that his material could not have been in a condition comparable to that used by others. Hyman's

TABLE 1

	NUMBER OF PIECES	HOURS	
		Distal Halves	Proximal Halves
June (room temperature).....	{ 35 18	42 53	52 73
December ($12^{\circ} \pm 2^{\circ}$ C.).....	{ 42 36	71 69	83 77

data for oral hydranths on distal and proximal halves of the same stems are given in Table 1.

In a more recent study Barth (1938a), taking volume of hydranth primordium divided by time from section to emergence of hydranth ($\pi r^2 L/t$) as the measure of rate of reconstitution, obtains much greater difference in rate at different stem-levels, since stem diameter and length of hydranth primordium decrease and length of time from section to emergence increases from distal to proximal levels. It seems open to question whether volume of hydranth primordium should be employed in a measure of rate in this case. Diameter of the primordium depends on diameter of the stem, which has nothing to do with reconstitution; and length of the primordium is not the result of growth but depends on the gradient length and scale of organization determined a few hours after section, before the primordium becomes visible (see pp. 101-2). Neither diameter nor length has anything to do with cell formation or growth; consequently their relation to rate of reconstitution appears at least doubtful. Driesch (1899b),

Morgan (1901*b*, 1903*c*), and Child (1907*b-e*) have shown that length of hydranth primordium differs in definite ways at different levels of section on oral and aboral ends of the same piece and in short pieces with difference in length of piece. These differences involve differences in localization and length of parts of the hydranth, that is, differences in scale of organization. Some of the measurements are given in order to show what actual lengths are found and how they differ. Driesch (1899*b*), using two successive pieces of *T. mesembryanthemum*, each 7-10 mm. in length, gave the following measurements: oral primordia of distal pieces, 1.67 mm., of proximal pieces, 1.21 mm.; aboral primordia of distal pieces, 0.96 mm., of proximal pieces, 0.9 mm. Measurements by Child (1907*b*), using two successive pieces 20-25 mm. in length, were: oral primordia of distal pieces, 2.26 mm., of proximal pieces, 1.33 mm. With three successive pieces, each 10 mm. in length, primordium lengths were as follows: oral primordia of distal pieces, 2.27 mm., of middle, 1.5 mm., of proximal, 1.3 mm. Aboral primordia of the same pieces were, respectively, 0.96, 0.85, and 0.91 mm. in length. The slight increase in length at the aboral end of the proximal piece probably results from some degree of physiological isolation of this region. With decrease in length of piece, length of oral and aboral primordia becomes, in general, more nearly equal; but even in pieces little longer than the two primordia the aboral may be shorter or less complete proximally than the oral. The proportions of the primordium, that is, the relative lengths of the four distinguishable zones (Fig. 13), also show a graded change from distal to proximal stem-levels, and the relation of primordium length to length of hydranth after emergence differs in the same way (Child, 1907*b, c*).

Differences in rate of development and length of primordium similar to those in *Tubularia* occur in *Corymorpha*, another gymnoblast hydroid, with difference in level of reconstitution (Child and Hyman, 1926). The hydranth of this species, however, develops gradually on the naked stem; and even during reconstitution contraction and extension occur; consequently, rate of reconstitution and length of hydranth primordium cannot be determined as exactly as in *Tubularia*.

The gradient pattern in a branching hydroid is very beautifully shown by *Pennaria cavolinii*, not only in the growth form but in the order of development of hydranths after their removal and the order of subsequent degeneration (Gast and Godlewski, 1903). Lateral branches in this species are oblique to the main axis and show a regular gradation in length basipetally. When all hydranths are removed, new hydranths develop basip-

etally in order from the apical region of the multiaxiate complex and from the apical end of each branch (Fig. 14, A). Later these hydranths degen-

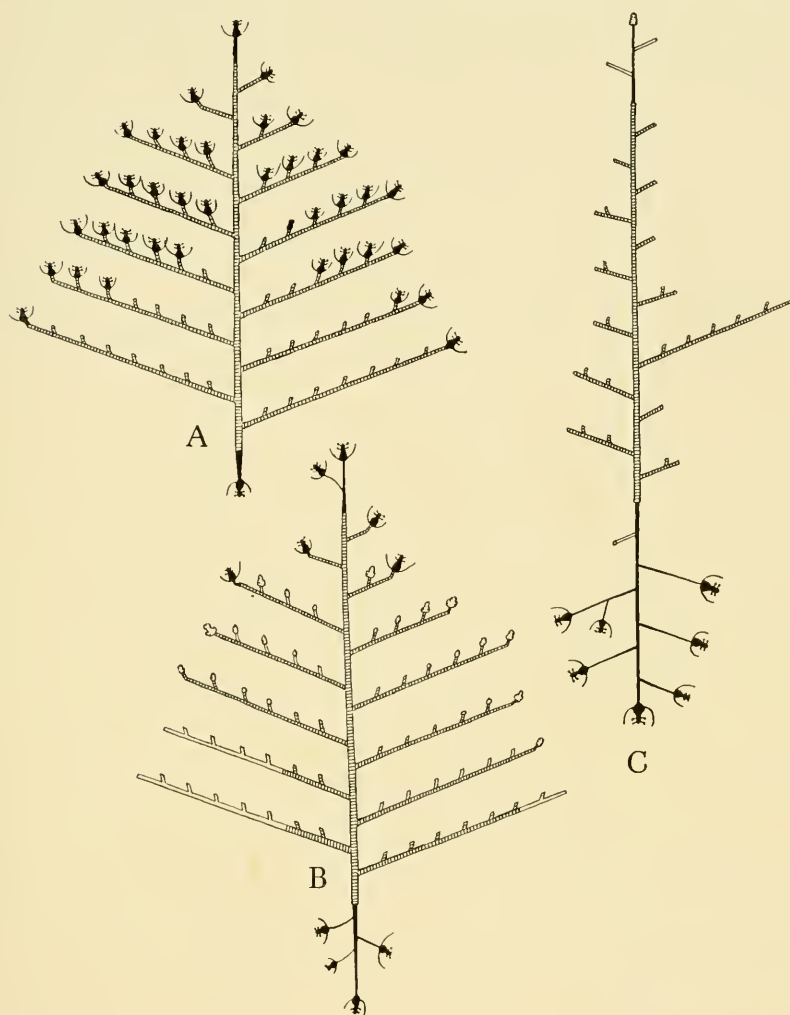


FIG. 14, A-C.—Reconstitution and degeneration of hydranths in *Pennaria cavolinii*. A, reconstitution of hydranths after removal progresses basipetally in the whole and in each axis; B, degeneration of hydranths and retraction of coenosarc progresses acropetally; C, the reconstituted axial system at the proximal end persists and grows longer than other parts (from Gast and Godlewski, 1903).

erate, retraction of the coenosarc in the branches occurs, and the empty parts of the perisarc drop off. These changes progress from the most basal

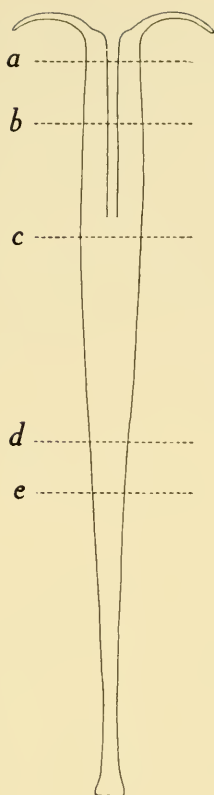
branches acropetally (Fig. 14, *B*). Meanwhile the original apical region of the main axis and the new multiaxiate individual which has developed from the proximal cut end may grow and give rise to new hydranths; evidently they are able to live at the expense of lower axial levels (Fig. 14, *C*). A gradient pattern is evident in this whole series of events. The rate of reconstitution decreases basipetally in the whole and in each axis; as the animals starve in the laboratory, lower axial levels are used in maintaining higher levels. Possibly differential tolerance to laboratory conditions is also involved (see chap. v). The reconstituted multiaxiate individual at the proximal end undoubtedly represents younger, more intensely active tissues than the old coenosarc, perhaps even more active than the original apical region, and so is able to persist and grow for a longer time than any other part. A few unpublished experiments with *P. liarella* indicate that

TABLE 2

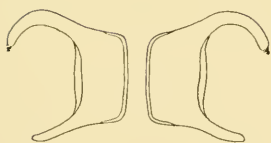
LEVELS	DAYS			
	3	8	14	26
<i>a</i>	1-2	7-10	20-25	12-20
<i>b</i>	0.5	6-8	10-15	12-15
<i>c</i>	0	5-7	5-10	12-18
<i>d</i>	0	0	2	10
<i>e</i>	0	0	0	0-5

a similar gradient exists in that form, though less regular, as the less regular growth form of the species would suggest.

A gradient in rate of reconstitution in *Obelia* has been noted by Billard (1904) and Lund (1923*a*) and in *Eudendrium* by Goldforb (1907). In the sessile scyphozoan *Haliclystus auricula* (Child, 1933*b*) and in the actinians *Cerianthus solitarius* (Child, 1903*a*), *C. aestuarii* (Child, 1908), and *Harenactis attenuata* (Child, 1909*a*) a similar gradient in rate occurs. Table 2 gives tentacle length for *Harenactis* pieces at different times after section at the five body-levels indicated in Figure 15, *A*. The tentacles from the more distal levels appear earlier, and in general grow more rapidly and attain greater length, than those of levels farther proximal. In the last column, 26 days, the tentacles of the *a*-pieces have begun to decrease in length, as all do sooner or later in animals kept without food and not permitted to burrow in sand. In these pieces the aboral ends were intact, and consequently the pieces differed greatly in length; but other experiments



A



B

FIG. 15

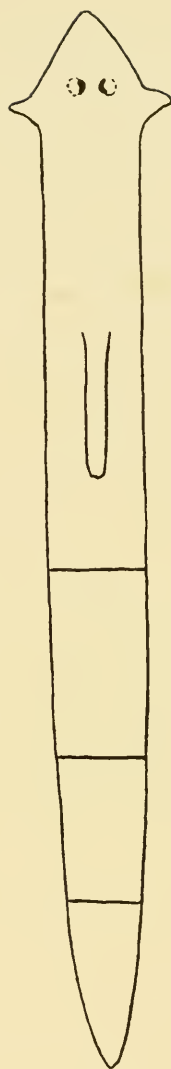


FIG. 16

FIG. 15, *A, B*.—*Harenactis attenuata*. *A*, outline indicating levels of section, *a-e* of Table 2; *B*, bipolar reconstitution from esophageal region; tentacles of disk at oral end develop more rapidly and become longer than those of aboral disk (from Child, 1909*a, b*).

FIG. 16.—Outline of planarian indicating approximate levels at which fission can be induced in long animals.

showed that length of piece became significant only in shorter pieces than those used here. Short pieces from the esophageal region often show bipolar reconstitution with development at the original oral end more rapid than at the aboral (Fig. 15, B).

Some studies of coelenterate reconstitution have been chiefly concerned with other points and give no information concerning differences in relation to body-level. Moreover, the original gradient differences in some of the more sensitive branching hydroids apparently decrease or even disappear under laboratory conditions, particularly if the animals are kept in standing water; hydranths degenerate and stolons develop in place of hydranths over the whole stock.

Although ctenophores are not now regarded as coelenterates, it may be noted here that no evidence of gradient difference has been observed in the reconstitution of *Mnemiopsis* (Coonfield and Goldin, 1937), although other evidences of an apical-oral gradient have been found (pp. 106, 327).

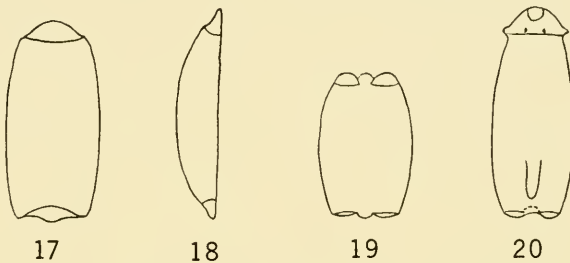
BODY-LEVEL AND RECONSTITUTION IN TURBELLARIA

Among the turbellaria the triclads are the most interesting forms in this connection because of the great capacity for reconstitution of certain species of the group and various limitations of this capacity in other species, because reconstitution in the group has been studied by many investigators, and because the gradient pattern is clearly evident in it.⁷

The described American species of *Dugesia* undergo fission at definite body-levels posterior to the mouth. The act of fission, which consists of an independent motor reaction of the posterior zooid region, is not usually preceded by visible morphogenesis but is a transverse rupture followed by reconstitution. This rupture, however, occurs at a definite body-level, and various lines of physiological evidence indicate that in animals above a

⁷ Recent taxonomic studies of American planarians by Hyman (1931) and Kenk (1935a) have resulted in extensive change in names of genera and species. According to Hyman, the genus *Planaria* does not occur in the United States, and the genus name *Euplanaria* replaced it for American species. However, it has recently been discovered by Hyman (1939) that the genus name *Dugesia* has priority over *Euplanaria*. This is an unfortunate discovery, for the names *Planaria* and *Euplanaria* are good descriptive designations and the anglicized "planarian" has definite meaning. Now American forms of the genus become *Dugesia*, while the European *Planaria* remains unchanged. The species distinguished by Hyman (1931) as *Euplanaria maculata* and *E. novangliae*, are, according to Kenk, a single species; and for this species the name *Dugesia tigrina* has priority, according to Hyman and Kenk. This species is regarded as including the form named *P. lata* by Sivičskis (1923); see also Watanabe, 1935b. *Planaria simplicissima* (Curtis, 1900) becomes *Curtisia foremanii*; *P. velata* becomes *Fonticola velata*; and the American dendrocoelid, often called *Dendrocoelum lacteum*, is *Procotyla fluviatilis* (Hyman, 1931). The revised nomenclature is used in the following pages.

certain length the posterior part of the body consists of one or more zooids, the number of zooids increasing with body length.⁸ Occurrence of a second set of sex organs in the posterior zooid is certainly rare but has been observed by Kenk (1935a) and is regarded by him as morphological evidence of the presence of a posterior zooid. Fission can be prevented or induced experimentally in *D. dorotocephala*. When it is prevented, animals 30–35 mm. in length, sometimes even longer, result. These give evidence of the presence of two, three, or more zooids, and fission can be induced at several levels (Fig. 16, *F*, *F'*, *F''*) of the postpharyngeal region (Child, 1910a). In the species which do not undergo fission, no evidence of any kind indicating presence of a posterior zooid has been found. Reconstitution in



FIGS. 17–20.—Planarian regeneration. Fig. 17, difference in rate at anterior and posterior surfaces of section and between median and lateral regions of a single surface. Fig. 18, more rapid regeneration ventrally than dorsally. Figs. 19, 20, regeneration in certain concentrations of Ringer solution without contraction of surface of section; cut ends of gut unite with ectoderm forming an opening into gut anteriorly and posteriorly; greater tolerance to Ringer anteriorly than posteriorly.

most planarians consists of more or less regeneration at surfaces of section with redifferentiation elsewhere, but in some it consists chiefly or wholly of regeneration.

As regards rate of reconstitution, it may be noted first that in isolated pieces, except when very short, regeneration in the earlier stages is more rapid at the anterior, than at the posterior, end of the piece (Fig. 17).⁹ Since this difference in early stages has no constant relation to the final amount of regeneration, it suggests the existence of a longitudinal differential or gradient in the planarian body. Early growth of new tissue is

⁸ Child, 1910a, 1911b, d, 1913b, 1930; Hyman, 1923b.

⁹ This difference has been observed in *D. tigrina* and *D. agilis* (Child, unpublished), in the form called *Planaria lata* by Sivičkis (1923), in a Japanese species resembling *D. dorotocephala* (Child, 1932b), in *Phagocata gracilis* (Buchanan, 1933), and repeatedly by groups of students in the study of reconstitution of *D. dorotocephala*.

also more rapid in the median ventral region than laterally and dorsally (Figs. 17 and 18), suggesting a differential from median ventral laterally and dorsally. These relations may be altered by incidental or experimental conditions; anterior or posterior outgrowth may be inhibited differentially (pp. 175-76). One example of some interest may be mentioned here. When pieces develop in approximately isotonic or hypertonic concentrations of Ringer solution, the cut ends do not contract, as they do in water; the cut end of the gut remains open and usually unites with the ectoderm at both ends of the piece, so that openings into the gut persist at both ends and the regenerating tissue is more or less divided (Figs. 19, 20). When level of section is through the pharyngeal pouch, its wall may unite with the ectoderm and so give rise to a terminal "mouth" with growth of new tissue on each side of it. The inhibition of median regeneration in these cases is evidently an incidental result of failure of the cut surfaces to contract in the salt solution and does not represent alteration in gradient relations. If the divided new tissue is removed and the piece returned to water, regeneration occurs as usual. Moreover, the wound does not remain open, as was maintained by J. W. Wilson (1926).

Rates of development of the head at different body-levels may be compared by determining the time between section and appearance of the black pigment of the eyespots. According to a statistical study (Watanabe, 1935a) rate of head development, as determined in this way, decreases from anterior to posterior levels as far as the fission zone and increases again in the posterior zooid region of *D. dorotocephala*. Other planarian species investigated show a similar relation between rate and body-level, except that in those that do not undergo fission the decrease extends to the posterior end of the body or as far posteriorly as head regeneration occurs.¹⁰ Rate of development of posterior new tissue shows a similar differential, slight in some species, strongly marked in others, except at extreme anterior levels; heads isolated by section at levels only slightly posterior to the eyespots heal posteriorly without further regeneration; sectioned at levels slightly farther posterior, they regenerate posterior ends incompletely or slowly. Posterior to these levels the minimum length of piece which reconstitutes a complete, normal individual increases posteriorly to the fission zone in species which undergo fission and is again less

¹⁰ Evidence of similar difference of rate has been found in the following: a European form called *P. dorotocephala* but probably another species (Pourbaix, 1931); a Chinese planarian (Li and Shen, 1934); *P. gonocephala* (Abeloos, 1930); *Dendrocoelum lacteum* (Sivičkis, 1931a, 1933); *Proctolyta fluviatilis* (Child, unpublished); *Phagocata gracilis* (Buchanan, 1933).

in the posterior zooid; in most other species it increases to the posterior end or as far posteriorly as head regeneration occurs, but even very small fragments of *Fonticola velata* from any postcephalic level reconstitute complete individuals, though only after encystment, practically complete degeneration of internal organs, and reorganization of body wall and parenchyma.¹¹

Marked differences in scale of organization of the preoral region occur in planarian reconstitution in relation to level of origin of the piece. These consist in a gradual decrease in relative length of the preoral region and more anterior localization of the pharynx in successive pieces of equal length from the most anterior level posteriorly to the fission zone, except in pieces from pharyngeal levels; in those the pharyngeal region or some

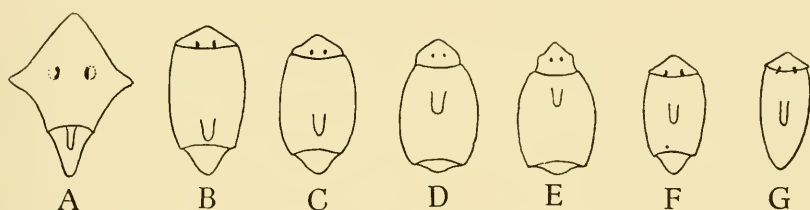


FIG. 21, A-G.—Differences in length of prepharyngeal region and in position of pharynx in pieces from different body-levels of *Dugesia tigrina*. A-E, decrease in prepharyngeal length in successive pieces from anterior to posterior end of anterior zooid; F, G, increase in prepharyngeal length in pieces from posterior zooid region.

part of it is already present, but its level may undergo gradual change in consequence of differential growth in relation to scale of organization (Fig. 21, A-E). In the region of the posterior zooid length of preoral region is again greater (Fig. 21, F, G). In species without a posterior zooid region decrease in preoral length continues to the posterior end. Pieces from anterior and posterior levels of *Curtisia foremanii* (Fig. 22, A, B) and similar pieces from young individuals of *Fonticola velata* reconstituting without encystment show this difference in scale clearly.¹²

Scale of organization of the new head in earlier stages shows a similar relation to level of origin of the piece. It decreases from the anterior level to the fission zone and is again larger in the posterior zooid region in the species with posterior zooids (Fig. 21). In those without such zooids the

¹¹ For fragmentation, encystment, and reorganization in *F. velata* see Child, 1913c, 1914d; W. A. Castle, 1928.

¹² For further data see the following: Morgan, 1898, 1900a, 1901c; Child, 1906, 1911b, 1913c, 1914d; Sivičkis, 1923; Buchanan, 1927.

decrease continues to the posterior end (Fig. 22). As these figures also show, the length of regenerated tissue posterior to the eyespots increases from anterior to posterior levels of the anterior zooid and is again less in the posterior zooid region (Fig. 21), or in species without posterior zooid it increases to the posterior end (Fig. 22). In other words, with increasing distance of level from the anterior end, an increasing proportion of the anterior region of the new individual is formed by regeneration rather than by redifferentiation, with decrease in regeneration again posterior to the fission zone when it is present. These differences in relative size and proportion of parts, as primarily localized and determined in pieces from different body-levels, gradually approach the normal in consequence of dif-

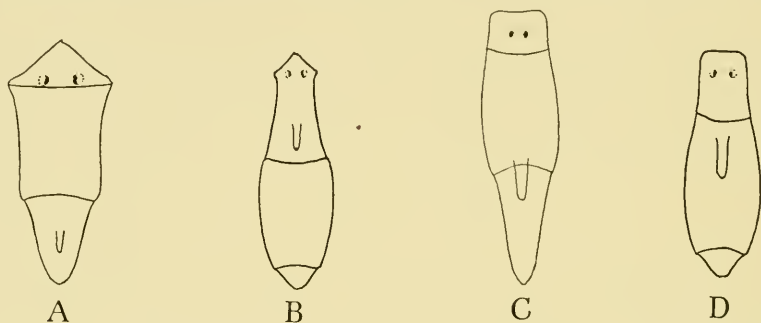


FIG. 22, A-D.—Differences in prepharyngeal length and position of pharynx in pieces from anterior and posterior levels. A, B, *Curtisia foremanii*; C, D, *Fonticola velata*.

ferential growth. Parts that are “too small” grow more rapidly than other parts in later stages, or in animals kept without food, at the expense of other parts with reduction in size of the whole. Changes in proportion are much more rapid in fed than in starved animals.

Certain planarian species show increasing inhibition of head development with decrease in length of piece below a certain fraction of body or zooid length. The length of piece at which inhibition becomes evident increases from anterior levels posteriorly or to the fission zone, but in the posterior zooid region it becomes very small. In pieces of equal length below a certain fraction of body or zooid length the degrees of inhibition increase from anterior to posterior levels, or to the fission zone, and are much less in the posterior zooid.¹³ The inhibited head forms and conditions which determine them are discussed in other connections (pp. 177-96).

The polyclad turbellarian *Leptoplana* does not reconstitute a head

¹³ For data see Child, 1911b; Child and Watanabe, 1935a; Watanabe, 1935b; Sivičkis, 1923, 1931a, b, 1933; Abeloos, 1930; Buchanan, 1933.

anteriorly from levels posterior to the cephalic ganglia but does regenerate posterior parts at all levels from a short distance posterior to the ganglia to the posterior end of the body (Child, 1904b). Both rate of growth and total length of posterior new tissue produced in pieces kept without food decrease from anterior to posterior levels. Rates and amounts of regeneration are considerably greater at any level when the cephalic ganglia are present than when they are absent. Pieces without ganglia are incapable of normal co-ordinated locomotion and show little motor activity. Apparently growth of the posterior regenerating tissue is stimulated in the normally active animal. Various rhabdocoel species show essentially similar conditions.

DATA FROM OTHER GROUPS

Some species of nemerteans show even greater capacity for reconstitution than planarians. According to Nusbaum and Oxner (1911), the slender form of *Lineus ruber* reconstitutes complete individuals at all levels; but pieces from the middle region have the highest rate. More recently Coe (1931) has found in *L. vegetus* and *L. socialis* in general a decrease in rate from anterior to posterior levels, but with considerable variation.

The many studies of regeneration in the annelids show very different degrees of regenerative capacity in different species. Briefly stated, the relation between regeneration and body-level in polychetes and oligochetes is, in general, as follows: In many forms studied the rate of regeneration at different levels has not been determined; but, according to the data at hand, rate of head regeneration, and in many species ability to regenerate a head, decrease from anterior to posterior levels. In some species a head is regenerated only at extreme anterior levels. Ability to regenerate a posterior end usually increases from extreme anterior levels over a certain portion of body length, which is very different in different species; and at more posterior levels rate of posterior regeneration decreases. In short, the differences in rate of regeneration at different body-levels in those forms which are capable of regeneration over most of the body length do not differ very greatly from those in planarians; but capacity for, and rate of, posterior regeneration usually increase over a greater distance from the anterior end than in planarians, though not necessarily greater in proportion to body length.¹⁴

¹⁴ See Hescheler, 1896; Morgan, 1897; Morgulis, 1907, 1909; H. R. Hunt, 1915; Hyman, 1916a; Korschelt, 1919; Sayles, 1934; and references cited by these authors. See also Sayles, 1940, *Biol. Bull.*, 78, 3.

Many annelid species regenerate no more than a certain number of segments anteriorly, even though more than that number have been removed. This number is characteristic for the species and may be regarded as representing a more or less specialized head region.¹⁵ Reconstitution by redifferentiation of some of the old segments posterior to the regenerated anterior end into more anterior segments replacing those which were removed above the number regenerated is known to occur in some species; but for others no data are at hand, though such reorganization of old segments is probably of very general occurrence. Here, then, as in planarians, reconstitution—at least in certain species—is in part regeneration, in part redifferentiation or reorganization of old parts. In some annelids, however, number of segments regenerated anteriorly is apparently not definitely limited but is more directly dependent on the number removed. In these cases there is probably less or no reorganization of old segments. Whether a difference in scale of organization in relation to body-level occurs in annelids does not appear from the data available, but an inhibition of head regeneration in relation to body-level and length of piece, much as in planarians, appears in *Lumbriculus* (Hyman, 1916a). As regards existence of a ventrodorsal or a transverse differential, it is found that outgrowth of new tissue from a cut end is, in general, more rapid in the median ventral region than elsewhere.

Relations between the different axes in certain of the branching bryozoan species resemble, more or less, those in the hydroids. When tips are removed from several branches at the same time, reconstitution begins first on the chief branch, also the ability to regenerate decreases basipetally in each axis (Otto, 1921).

In most other animal phyla the capacity for reconstitution is usually more narrowly limited. Asteroid starfishes regenerate arms, and a few species are able to regenerate disk and other arms from a single arm without any portion of the original disk. Each arm develops like a new polar axis from the tip basipetally, and outgrowth of new tissue is more rapid in the median ventral region than elsewhere; that is, a longitudinal and ventrodorsal gradient are indicated in development of the arms. Rate of regeneration of larval legs of the insect *Cloe diptera* decreases from anterior to posterior levels (von Ubisch, 1915). Attention directed to this point would perhaps show similar differences in other arthropods. Some of the ascidians are capable of extensive reconstitution. Pieces of *Clavelina* from

¹⁵ Morgan, 1897; Hyman, 1916a; Gross and Huxley, 1935; Berrill, 1936a, b. See also Hyman, 1940, *Amer. Nat.*, 74, No. 755.

the "esophageal" region proximal to the branchial chamber develop into bipolar forms with a branchial chamber at each end, but distal development is more rapid than proximal (Brien, 1930; Pasquini, 1933).

THE QUESTION OF REGENERATION IN RELATION TO DEGREE OF INJURY

Increase in rate of regeneration with increase in size or number of parts removed has been reported for certain forms and has raised the question whether increase in degree of injury increases the rate.¹⁶ In this connection it is of interest to inquire whether the differences in rate of reconstitution at different levels of the longitudinal axis noted earlier in this chapter are similarly related to degree of injury. Rates of hydranth reconstitution in hydroids and of apical reconstitution in other coelenterates and rate of head regeneration in planarians and annelids decrease from anterior to posterior levels, that is, with increase in size of the part removed anteriorly, obviously an increase in degree of injury. Evidently this increase is not concerned in these cases in determining rate of regeneration, for the rate is highest when the injury is least. Posterior regeneration in planarians and annelids is usually absent, incomplete, or slow at levels close to the head, that is, when degree of injury is greatest; at levels a little farther posterior, with a lesser degree of injury the rate is highest and decreases from these levels posteriorly. This decrease might perhaps be regarded as related to decrease in degree of injury if head regeneration did not decrease in rate in the same direction with increasing degree of injury anteriorly. If rate at either end is affected by degree of injury at both ends, little difference in rate in pieces of equal length from different levels should occur, for degree of injury is much the same in such pieces; if the part removed anteriorly is small, the posterior part removed is large, and vice versa. In short, these differences in rate at different levels of the longitudinal axis appear to be related to inherent graded differences in physiological pattern at different levels rather than to degree of injury. Moreover, the scale of organization of the oral hydranth in *Tubularia* and *Corymorpha* is largest when the degree of injury at the oral end of the piece is least, and that of the aboral hydranth is largest when degree of aboral injury is greatest. It appears probable that in cases of apparent relation between rate of regeneration and degree of injury, for example, the increase in rate in the starfish with removal of an increasing number of arms, the increasing disturbance of function, is a stimulus to regenerative growth.

¹⁶ Zeleny, 1905*a*, *b*, 1909; Ellis, 1907, 1909.

RECONSTITUTION AFTER SECTION OBLIQUE TO THE LONGITUDINAL AXIS

Differences in rate of reconstitution in relation to different levels of a cut end oblique to the longitudinal axis have been observed in a number of coelenterates and planarians. When stem pieces of *Tubularia* or *Corymorpha* are cut with plane of section strongly oblique to the longitudinal axis, the distal tentacles of the hydranth often develop first at the

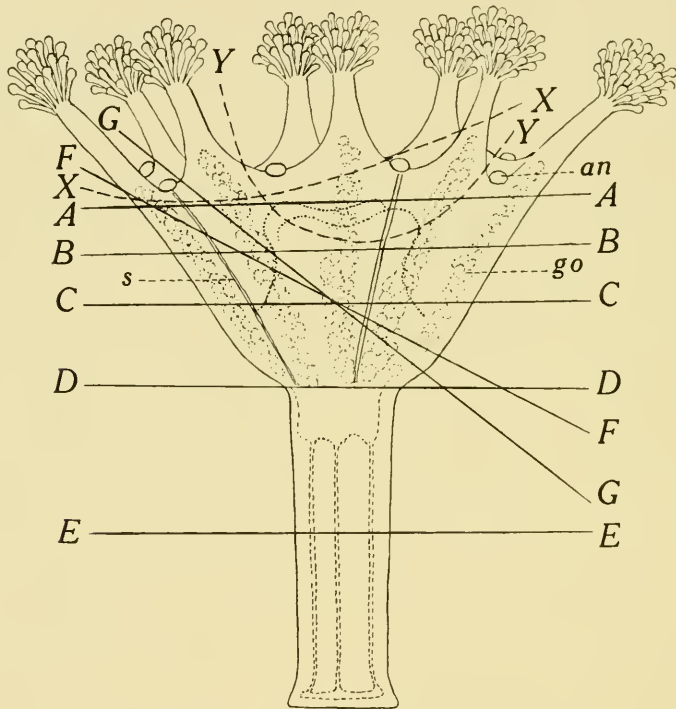


FIG. 23.—*Haliclystus auricula*. Lines AA-GG, XX, FF, indicate levels and directions of section (from Child, 1933b).

most oral region and progressively to lower levels, forming a ring of distal tentacles oblique to the polar axis of the stem. In these forms, however, closure of cut ends is rapid, and the oblique surface may become rounded before tentacles develop. When this occurs, distal tentacles develop near the oral end as usual, but the plane of the tentacle ring is transverse, not oblique.

Difference in rate in relation to an oblique cut surface appears much more clearly in the sessile scyphozoan *Haliclystus*. After transverse section at various levels of umbrella or stalk (Fig. 23, AA-EE) reconstitution

of the distal part removed occurs as indicated in Figure 24, *A* and *B*. If the plane of section is strongly oblique to the longitudinal axis (Fig. 23, *FF*), reconstitution occurs most rapidly on the most distal part, and the rate decreases proximally until at the most proximal levels nothing more than wound-healing may occur (Fig. 24, *C*). If the oblique section is made so that one or two of the original marginal organs remain (Fig. 23, *GG*), reconstitution of marginal structures may be largely or even com-

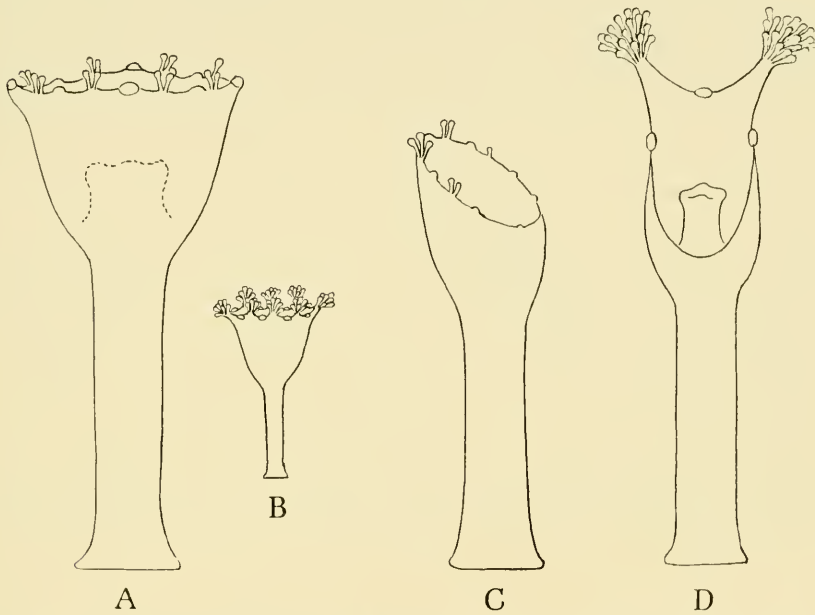
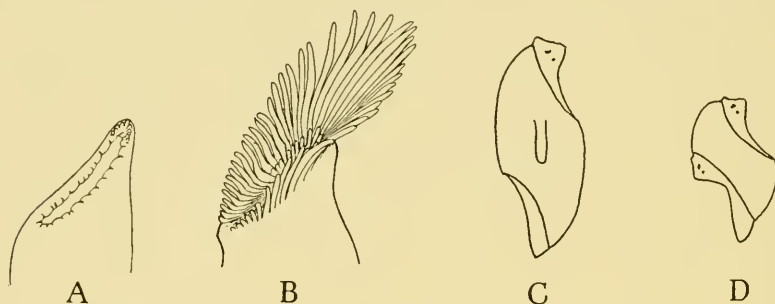


FIG. 24, *A-D*.—Reconstitution in *Haliclystus*. *A*, early stage after transverse section at levels *BB* or *CC* of Fig. 23; *B*, advanced stage after section at level *EE* of Fig. 23; *C*, early stage after oblique section with removal of all marginal organs; *D*, reconstitution after oblique section leaving small part of original margin (from Child, 1933*b*).

pletely inhibited (Fig. 24, *D*). Later removal of the original marginal parts, even after a week or two, is followed by development of at least some of the marginal structures previously inhibited. When only a small part of the marginal region is removed, as indicated by *XX* and *YY* in Figure 23, little or no development of the parts removed occurs (Child, 1933*b*).

After oblique section of the scyphistoma of *Aurelia* the rate of reconstitution decreases from distal to proximal levels of the oblique surface, as in *Haliclystus*. Tentacles from the most distal level attain full length in a

few days; but, although tentacle development is not completely inhibited at the most proximal levels, the full normal number, sixteen, is not usually attained, even after 3 or 4 weeks, and the tentacles farthest proximal appear last and develop very slowly. Experiment on another species of stalked scyphozoan (*Thaumatoscyphus distinctus*) shows a delay of reconstitution at proximal levels of an oblique cut surface more nearly like that in the scyphistoma than in *Haliclystus* (Hanaoka, 1935). In these forms there is not only a difference in time of appearance of marginal structures at the different levels of the oblique section but a continuing dominance of the distal region which greatly retards reconstitution at the more proximal levels or, in *Haliclystus*, completely inhibits it. Similar



FIGS. 25, A-D.—Reconstitution after oblique section. Fig. 25, A, early, and B, late, stages of distal reconstitution in *Cerianthus solitarius*; C, anterior and posterior planarian regeneration after oblique section; D, development of both head and posterior end on oblique posterior cut end of short piece.

differences in rate on an oblique cut end appear in the actinians *Cerianthus solitarius* (Child, 1904a) and *C. aestuarii* (Child, 1908), as indicated in Figure 25, A and B. In both species development at the most distal level is more rapid, and that at the proximal level less rapid, than on transverse cut surfaces at corresponding levels.

On strongly oblique anterior cut ends of planarian pieces head regeneration is localized on the more anterior part; and the more anterior side of the head, including the eyespot, usually develops more rapidly than the other side (Fig. 25, C, D). This asymmetry constitutes further evidence of difference in rate of head development at different body-levels. A regenerating posterior end is localized on the more posterior part of an oblique posterior cut surface (Fig. 25, C). The posterior end is a subordinate part and is determined by levels anterior to it (pp. 339-40). Physiological dominance of more anterior levels is undoubtedly concerned in

determining its position on the oblique surface. That this is the case is further indicated by occasional development of a head on the more anterior part of the posterior, as well as of the anterior, oblique end in short pieces and more rarely of both head and posterior end on the posterior oblique surface (Fig. 25, *D*).¹⁷

In the reconstitution of short pieces half, or less than half, the width of the planarian body the head may develop on the angle between anterior and median cut surfaces or entirely on the median, instead of on the anterior, surface. The longitudinal axis thus determined is oblique or vertical to the original anteroposterior axis. In these heads the eyespot of the side nearest the original anterior end often appears first, and the whole head may be asymmetrical in earlier stages.¹⁸

VISIBLE EVIDENCES OF PATTERN IN EARLY EMBRYONIC DEVELOPMENT

The earliest indications of embryonic order and pattern are usually regional differentials or gradients of one kind or another—of amount of yolk, visible character and staining of the cytoplasm of the egg or cleavage stages, rate of cell division, size of cells, rate of growth, rate of differentiation, etc. Some of these evidences of pattern—yolk gradients, for example—are present in undivided eggs of some species; others appear during cleavage or later; still others, only as particular organ systems begin visible development. Very commonly pattern is first evident along what becomes the polar axis and becomes evident in other directions corresponding to other axes as development proceeds. However, some eggs exhibit, at or before the beginning of development, more or less definite regional differentiations in the cytoplasm which are, or become, features of embryonic pattern. So-called “mosaic development” furnishes numerous examples of this type of pattern (see chap. xiv). Such eggs may be, and now usually are, regarded as having already undergone a greater or less degree of regional cytoplasmic differentiation which becomes the basis of embryonic differentiations. Since these differentiations are, or become, definitely related to axiate embryonic pattern and since they occur only in certain species, they probably represent derivatives, effects, expressions of pattern, rather than the primary pattern itself. But whatever the condition of the egg cytoplasm at the beginning of development,

¹⁷ These oblique reconstitutions in planarians have been repeatedly described. See, e.g., Morgan, 1900, 1901c, 1904b; Bardeen, 1902; Rand and Boyden, 1913. They have been used by the writer as class experiments for many years.

¹⁸ Morgan, 1900, 1901c, 1904b; Child, 1915c, p. 164; Olmsted, 1918; Beyer and Child, 1930. See also pp. 365–66.

gradients in rate of cell division, in size of cells, and in rate of growth and of differentiation appear in relation to the axes of the whole and of parts as development progresses.

The very general occurrence of an anteroposterior developmental gradient has been recognized in the so-called "law of anteroposterior, cephalocaudal, or craniocaudal development."¹⁹ But developmental gradients equally definite in character and equally constant in occurrence appear in other directions—for example, from the ventral region laterally and dorsally in turbellarians, annelids, and arthropods and from the mid-dorsal region laterally and ventrally in vertebrates. In certain other groups—coelenterates, trematodes, cestodes, nemerteans, nemathelminths, mollusks, and echinoderms—an apicobasal or longitudinal developmental gradient appears more or less clearly, at least in the egg, in cleavage or in larval stages; but other gradients differ according to developmental pattern in different members of these groups. As development proceeds, new gradients may arise at various levels and in various directions with localization of organ primordia. Every such localization alters, and may even obliterate or reverse, the pre-existing gradient or gradients. Development of the first hydranth from the originally basal end of the planula of calyptoblast hydroids with reversal of the original polarity is an example (pp. 96, 97). Many axiate organs and organ systems make their appearance as localized areas or fields of developmental activity in which the activity decreases from a region more or less centrally or otherwise localized in the area. A tentacle, for example, usually begins its development as a region in which developmental activity decreases more or less radially from a center and becomes axiate in consequence of greater growth of the central region; its developmental pattern is essentially like that of other buds. Conditions in early developmental stages of many appendages are apparently similar, but the most active region is not necessarily at the geometrical center of the field. In short, observation alone gives evidence of gradients and gradient systems appearing and undergoing change in definite order and relation as characteristic features of earlier embryonic developmental stages of many organisms and organs. Gradients appear to precede sharply defined and bounded morphogenetic differentiation.

Even in advanced stages of development, in which the changes consist

¹⁹ Morphologically, this gradient is merely a timetable of events, but there must be a physiological basis for this timetable. In this connection see Kingsbury, 1924, 1926, 1932; Child, 1925c.

largely of growth in size, growth gradients appear in many parts as either absolute or relative differences in growth rate. Huxley's recent quantitative study of relative growth is of interest in this connection (Huxley, 1932).²⁰ Growth gradients and allometric growth occur very generally in the course of development. Allometric growth, that is, growth of a part at a different rate from growth of the whole, appears almost universally in the apicobasal or longitudinal axis at some stage of development. In the hydroids *Tubularia* and *Corymorpha* length of hydranth in early stages may be half or more than half the total body length, but both absolute and relative lengths of stem increase until in large individuals stem length is many times hydranth length. Evidently the stem grows in length more rapidly than the whole. In planarians also, relative growth in length with increase in total length increases from anterior to posterior levels (see, e.g., Abeloos, 1928). Embryologically the pharynx arises near the posterior end, but in long individuals it lies anterior to the middle. This higher growth rate of posterior regions is most conspicuous in those species which develop a posterior zooid or zooids and undergo fission; but in these forms it is not, properly speaking, growth of a single individual. Such growth gradients do not necessarily correspond in direction with the primary gradient of the longitudinal axis, and certain characteristics of the primary gradient may persist independently of growth gradients of later stages. In *Tubularia* and *Corymorpha* the high end of the gradient, indicated by respiration, differential susceptibility, and reduction of vital dyes, is the hydranth; but it is the low end of the gradient of longitudinal growth in postembryonic stages. In fact, growth is very often greater at the lower than at the higher levels of gradients, indicated by differences in respiration and various other physiological factors.²¹ In general, the correspondence between these gradients indicated by different methods appears to be closer in earlier than in later stages of development. Undoubtedly, gradients characterized by other activities than growth occur. In fact, higher growth rate at lower levels of such gradients may occur because other activities are less intense there.

Graded morphological differences corresponding to the physiological gradients present often appear, particularly in the longitudinal axes, in adult animals. Distribution of setae, pigmentation, and nephridia of cer-

²⁰ Huxley and Teissier (1936) have suggested that the term "heterogony," used by Huxley (1932), be replaced by the term "allometry."

²¹ In his discussion of gradients Needham (1931, pp. 582-606) has apparently failed to grasp this point, that a gradient indicated by differences in respiration, susceptibility, etc., does not necessarily correspond to a growth gradient in the same axis.

tain oligochetes show such graded differences along the anteroposterior axes of certain oligochetes coinciding with the physiological gradients present (Pickford, 1930; Sivičkis, 1930). The functional activities of various organs also give evidence of the presence of physiological gradients of some sort. Transmission of impulses may occur more rapidly, or with greater range, in one direction than in the reverse, as in the ctenophore plate row (Child, 1917*c*, 1933*a*). Isolated pieces of the mammalian intestine show a gradient decreasing posteriorly in rate of rhythmical contraction and a gradient in the same direction of excitability and various other physiological characteristics (Alvarez, 1928).

CONCLUSION

It has already been noted that buds provide material much more accessible than the ovarian oöcyte for obtaining information concerning the beginnings of developmental pattern. Development of an individual from a bud is physiologically one of the most interesting and important forms of development occurring under natural conditions. Bud pattern may originate in a single cell or in a cell group which has previously constituted a part of an individual, and cells from different parts of the individual may give rise to buds. They have not had the same past history, nor have they developed an organization, a pattern, like that of the egg. As far as they are concerned, the new pattern may be entirely accidental: they merely happen to be in the locus of the activation. The transformation of the radial gradient system primarily characteristic of buds into an axial system by differential growth is highly suggestive for the problem of axiate pattern. Is the physiological polarity which develops from the primarily radial pattern anything more at its inception than an altered spatial order of the radial gradient system?

Fissions involve reconstitution with either modification of a pre-existing partial pattern or development of new pattern; but they have the disadvantage, for certain experimental purposes, that they usually occur only in certain regions of the parent body and under certain conditions, though it is sometimes possible to induce them experimentally. The reconstitutions following section can be initiated at will with variation of form, size, region or origin of the isolated part, and physiological condition of the parent individual, as affected by age or nutrition or as altered experimentally. Also, the effect of experimental conditions on developmental pattern of isolated pieces of different origin and in different physiological condition can be investigated. Only certain directly distinguish-

able characteristics of reconstititional patterns have been considered in this chapter, but these have brought to light certain points of interest.

It is evident that, even though they finally become similar individuals, the patterns of reconstitution in pieces from different body-levels are primarily different and that these differences are, in general, graded in character and indicate graded differences of some sort in the parent body. Except for failure of extreme apical or anterior regions to reconstitute more basal or posterior parts and absence of head regeneration at the more posterior body-levels in certain species, the differences in pattern are quantitative, rather than qualitative or specific, differences in rate, size, length, and proportion rather than in the kind of organs which develop. These facts suggest that the differences are expressions of essentially quantitative differences along the parent axes, whatever the specific organ differences which may be present at different levels. If we admit this, it follows that these quantitative differences in the parent body are essential factors in determining developmental pattern in the isolated pieces.

Gradations or gradients of some sort, whose presence is indicated in one way or another in development under natural conditions, are characteristic of embryonic, as well as of other developmental, patterns. Consideration of the question of their significance in embryonic pattern and of their possible relations to the apparently specific regional differences in egg and embryo, which are revealed by experimental procedures, is postponed until other experimental data bearing upon the problem of pattern have been presented.

CHAPTER III

CONCERNING METHODS OF PHYSIOLOGICAL ANALYSIS

IN THE attempt to throw light on the problems of development many methods have been developed and employed and have given a large body of evidence. Since the mature organism is the result of development, it, as well as earlier stages, has been used as material in attacks on problems of development, but the data obtained are of interest chiefly in the light of what is learned from earlier stages. Some consideration of these methods, their objects, their limitations, and particularly some of the difficulties involved and the precautions necessary appears desirable as a preliminary to discussion of results obtained with them.

METHODS FOR DETERMINING RESPIRATION

Since the axial developmental gradients appear to involve protoplasmic activity of some kind and since respiration, as determined by oxygen consumption and carbon dioxide production, is a measure of certain protoplasmic activities and, to greater or less degree, an indicator of others, the question at once arises whether differences in rate of respiration bear any relation to the gradients or to other features of developmental pattern. Unfortunately, most of the methods available at present for direct determination of respiratory rate are rather unsatisfactory for the purpose of determining whether such differences exist, because they make it necessary to separate different regions of the body and to determine respiration in the isolated pieces. This procedure introduces various difficulties and complicating factors, some of which cannot be entirely overcome or avoided; but if they are not considered and eliminated or controlled as far as possible, the results obtained are without definite significance. When an organism, particularly an animal, is separated into pieces for determinations of respiratory rate of different regions along an axis, several questions arise: First, is respiratory rate altered in consequence of section? Second, if alteration occurs, is the rate increased or decreased? Third, are pieces from different levels of the axis affected to the same degree by section? Fourth, if alteration of rate occurs after section, is it persistent or temporary? Fifth, if the alteration is temporary, does the rate sooner or later

become the same as that of the region of the intact body which the piece represents, or does it remain above or fall below that? Sixth, does loss of body fluids or blood in consequence of section affect the respiratory rate of pieces? Seventh, in pieces which undergo reconstitution is respiratory rate altered by the reconstititional activities, and, if so, how soon after section does this alteration occur? Eighth, if pieces are motile, is motor activity of pieces from different body-levels different in degree, and, if it is, to what extent does this affect respiration? In certain cases the question of occurrence or of intensity of anaerobic respiration must also be considered.

At present it is not possible to answer all these questions with certainty. For example, we cannot be entirely certain whether the respiratory rate of an isolated piece from a certain body-level is the same as when the piece was a part of the intact body; nor can we determine with certainty when reconstititional activity begins to affect the respiratory rate, although it appears beyond question that it does increase the rate. As a matter of fact, in most animal species thus far investigated the respiratory rate is undoubtedly increased, sometimes very greatly, immediately following section and gradually decreases during one to several hours following; the rate of all the pieces together may finally become about the same as that of the animal before section, but we have no means of knowing that the rate of each piece is the same as when it was part of the intact body. The total rate of pieces of some other animals may fall below that of the intact animal; consequently, the significance of differences in pieces from different levels becomes still more uncertain. Motor activity presents another difficulty in motile animals. In planarians, for example, pieces above a certain fraction of body length from anterior regions of the body show, in general, more motor activity for some time after section than pieces from more posterior levels. These differences, however, can be practically eliminated by decreasing length of pieces, controlling illumination, and providing conditions favorable to aggregation of the pieces. In naked-bodied aquatic forms which can withstand only brief exposure to air, weighing of pieces presents certain difficulties. Some forms secrete considerable slime when exposed to air, and transfer connected with weighing may also increase slime production. If perisarc or other skeletal substances are present, they usually differ in amount at different body-levels and become a factor in weight.

Elongated organisms of considerable size, at least several millimeters in length, and without great regional differences are the most favorable

material for determinations of difference in respiratory rate along the polar axis, but difficulties and more or less uncertainty are involved in all such determinations. Such organisms are either fully developed or in an advanced stage, and data from them can be no more than suggestive or indicative of conditions in earlier developmental stages. They give no information concerning axial characteristics at the beginning of development or of changes which may have occurred during its course. Moreover, some of them, such as planarians and annelids, have certain organs at particular body-levels which may have respiratory rates different from those of other parts of the body at the same level; also, differences in rate along the gut may not be in the same direction as differences in the body wall. In planarians differential susceptibility and rate of dye reduction in the body wall decrease from the head posteriorly to the fission zone in forms with a posterior zooid, but there is some evidence that the gradient in the gut decreases in both directions from the pharyngeal region. If respiratory rates parallel these differences, the respiratory rates of pieces from different levels of the planarian body may not show the differences which are actually present, because differences in the gut may more or less balance differences in the body wall. In practice we may apparently decrease differences in the gut by starving the animals for a week or more, but even with this precaution we cannot be certain that we obtain a true picture of the respiratory differentials in the body; nevertheless, the fact that differences in rate are usually absent in pieces of animals with food in the gut and become definite and consistent in sufficiently starved animals appears in some degree significant (pp. 108, 737). The unbranched stem (hydrocaulus) of the hydroid *Tubularia*, or *Corymorpha*, meets the desired conditions, as far as differentiation of regions is concerned, as nearly as any animal species; but in the case of *Tubularia* the presence and different thickness of the perisarc at different levels and in *Corymorpha* the increase in volume of the entodermal parenchyma basipetally and the presence of perisarc on the basal part of the stem show that even these forms are not without possible complicating factors for respiratory determinations (Child and Hyman, 1926; Hyman, 1926a).

Determinations or estimations of CO_2 production also give some information concerning regional differences in respiratory rate, but essentially the same difficulties are involved as with determinations of oxygen consumption.

The chief methods available for determinations of oxygen consumption bearing upon the question of a respiratory pattern in the individual are the

Winkler method and the respirometer and microrespirometer methods. The Winkler method, as used for aquatic respiration, consists in determination of the amount of oxygen dissolved in water, before and after a period of respiration, by the material concerned in a closed container without air. The procedure consists in the addition of the Winkler reagent to a known volume of water and titration. With the respirometer methods the quantity of oxygen consumed is determined from decrease in gas volume measured in the manometer connected with the respiratory changer, the carbon dioxide produced being removed. When the material is aquatic, it is usually placed in a small volume of water in the respiratory chamber, and provision is made for maintenance of equilibrium of oxygen in air and water in the chamber, usually by some type of shaker. Constant temperature is maintained.

The Winkler method, as used by Hyman and others with various invertebrates,¹ has been criticized on the ground that absorption of iodine by substances in the water, particularly organic substances, such as slime or tissue fluids, may result in incorrect oxygen values.²

Carbon dioxide production can be determined or estimated in various ways. The first data on differences along the polar axis were obtained with planarian material by Tashiro with the "biometer" (Child, 1913a), but most of the later work along this line has been done with colorimetric methods. A method of comparative estimation used with *Dugesia dorotocephala* (Robbins and Child, 1920) and with *Corymorpha* (Child and Hyman, 1926) is based on color change of a nontoxic acid-alkali indicator (phenolsulphonephthalein) in solution in the water containing the pieces, equal weights of animal material and equal volumes of solution being used for comparison. The rate of change in hydrogen-ion concentration, as indicated by change in color of the indicator solution or the time required to attain a certain color as compared with standard colors of known pH, serves for comparison of different lots of material. In another colorimetric method³ the material, in a small volume of water, if aquatic and unable to stand exposure to saturated air, gives off CO₂ into the water; it passes from the water into the air of the respiratory chamber and from the air into the indicator solution. Equilibrium between air and indicator solution is maintained by continued to-and-fro movement less rapid than the

¹ Hyman, 1916b, 1919a, b, c, d, e, 1920a, c, 1923b, 1925, 1926a, 1932a; Hyman and Galigher, 1921; Child and Hyman, 1926; Lund, 1928.

² See Appendix I, pp. 729-31.

³ Parker, 1925, 1929; Watanabe, 1931; Watanabe and Child, 1933.

usual shaking.⁴ Neither of these two methods provides for the rather remote possibility of production of other gaseous or volatile acid-forming substances or for production of ammonia.

As regards the problem of developmental pattern, it is much more important to know whether a respiratory pattern is present in eggs and early developmental stages, and what changes it may undergo during development, than to determine whether such pattern is present in adult individuals; but the difficulties are even greater. Although the data thus far obtained with adult forms give no information concerning earlier stages, they are of some interest to developmental physiology because, in those cases in which evidence of a respiratory gradient has been obtained, this gradient parallels gradients indicated by other methods, and in some forms these other gradient expressions persist from early development throughout life, at least in certain parts of the body. Gradient patterns indicated by differential susceptibility, differential dye reduction, and in other ways are characteristic of early developmental stages; and their coincidence with respiratory gradients in fully developed individuals suggests the possibility that a respiratory gradient pattern may also be present in earlier stages of development. Granting the possibility of sufficiently accurate determination, however, separation of the unfertilized or fertilized egg into pieces leaves the nucleus in one piece if it is not destroyed or injured, although in many eggs fertilization of the nonnucleated piece is possible. Separation of blastomeres and of parts of embryos and larval stages is possible, but the same questions as to effects of separation arise as with pieces of adult animals, and the possibility of periodic change in respiratory rate in connection with the cell-division cycle must also be considered. As development progresses, localization of organs and appearance of new axes may introduce further complication. Respiration of parts of amphibian embryos isolated by section has been determined by J. Brachet (1934*a*, *b*, *c*, 1936), and more recently determinations have been made on different regions of intact unfertilized eggs and embryos by introduction of a single egg or embryo into a capillary tube with diameter of lumen equal to, or slightly smaller than, that of the egg and connection of both ends of the tube with a microrespirometer (J. Brachet and Shapiro, 1937). With this method the organism is not injured in any way, and oxygen uptake of two opposed sides can be determined separately. Results obtained in this way are discussed in the following chapter. Still

⁴ See Appendix I, p. 731.

more recently studies on respiration and glycolysis have been made on small fragments of amphibian embryos with the Cartesian diver "ultra-microrespirometer" (Boell *et al.*, 1938, 1939).

In so far as they involve separation of the material into pieces, the methods for determining regional differences in glycolysis present the same difficulties and raise the same questions as those for oxygen consumption and CO₂ production.

DIFFERENTIAL REDUCTION OF POTASSIUM PERMANGANATE

Potassium permanganate is a powerful oxidizing agent and is readily reduced by living protoplasts to oxides of manganese, which are precipitated and stain the protoplasm brown or, in high concentration, opaque black. As might be expected, this substance is highly toxic and may be used as a lethal agent to show death gradients. In very low concentrations, however, intracellular reduction and coloration of the protoplasm take place slowly; and axial differentials in rate and, in some cases, in depth or density of coloration can be observed. Concentrations used in this way range from $m/2,000$ to $m/100,000$, according to material and rate of coloration desired. The method is chiefly of value for small more or less transparent forms, such as protozoa, some eggs, blastulae, planulae, and gastrulae, but may also be used to show differential reduction in the ectoderm of hydroids, medusae, and various larval forms and on cut surfaces at different levels of an axis. Even small embryonic or larval stages of some species become opaque black if the reaction is allowed to continue to completion and final death of all parts. In some of these, however, the organism becomes more or less translucent after dehydration and clearing, and a gradient in depth of color is visible; but in the clearing oil or in balsam mounts the color gradually disappears. It is perhaps scarcely necessary to point out that observation of the color through different thicknesses of tissue in the same organism may lead to incorrect conclusions. In organisms killed by boiling water or by various fixing agents the amount of reduction, as indicated by depth of color, and the rate of reduction are greatly decreased; and either no gradient appears or a slight gradient is present immediately after killing but soon disappears.

The gradient in rate of reduction might conceivably result from a gradient in rate of penetration of KMnO₄, but evidence indicating appreciable differential in penetration has not been obtained. The reduction color is at first uniform, as reduction occurs superficially in the cell or cells, and

the color gradient appears only gradually. If any permeability gradient were originally present, it is probably destroyed at once.⁵

DIFFERENTIAL INTRACELLULAR INDOPHENOL REACTION

The indophenol blue reaction between α -naphthol and dimethyl-paraphenylenediamine, which is catalyzed by certain oxidases, has been used with various ciliate protozoa, embryonic and larval stages, and small animals, also in certain experiments on reconstitution. A very distinct axial gradient in appearance within the cell or cells of the indophenol blue color is characteristic of the living animal, but none or only traces for a short time if the organisms are first killed. The reagents must be used in extremely dilute solutions, to avoid killing. The extensive literature on the indophenol reaction, the indophenol oxidase or oxidases, and their relation to tissue oxidation and respiration need not concern us here. It has been shown that dimethyl-paraphenylenediamine increases oxygen consumption in certain tissues and that α -naphthol inhibits oxidations. Moreover, not all the oxidation products of paraphenylenediamine give indophenol blue with α -naphthol, and it may happen that the more vigorous the oxidation, the less blue is formed. Also, indophenol blue may be decolorized by the reducing action of certain tissues. The greater part of the work with this reaction has been done with vertebrate tissues, and it is by no means certain that the conclusions and hypotheses based on it hold without modification for the lower invertebrates, embryonic tissues, and unicellular organisms.

Attempts have been made with hydroid planulae and starfish blastulae and gastrulae to determine whether or to what extent differential permeability to the agents might be concerned. The material was placed first in one of the agents for one to several minutes, then in the other. It was found that with this procedure both agents, in the very low concentrations used, were present in all parts of the material within a short time in sufficient quantity to give the same reaction as with much longer exposure. Five minutes or less was sufficient for complete penetration of either reagent, but difference in time of the reaction along a polar axis may be an hour or more. That the indophenol gradient indicates an axial physiological differential of some sort is evident; that it indicates a differential associated with indophenol oxidase appears highly probable; and that it

⁵ For use of KMnO_4 in this way and results obtained see Child, 1919a, f, 1921a, 1925a, 1926a; Hyman, 1920b; Galigher, 1921a.

represents one aspect of an axial differential which expresses itself in various ways is indicated by the fact that it parallels gradients shown by other methods in the same material.

Benzidin has been used as a test for peroxidase activity in sea-urchin eggs and embryos;⁶ but since the method involves killing the material, the significance for the living organism of observed differences or absence of difference remains a question.

DIFFERENTIAL STAINING BY VITAL DYES

The so-called "vital dyes" which stain intracellular constituents in the living organism are more or less toxic and, with sufficient staining, kill. Certain of these dyes undergo reduction in living cells under anaerobic conditions or in low oxygen, becoming colorless (methylene blue, etc.) or changing color in the course of reduction (Janus green). Use of dye-reduction methods is considered in the following section. For the present, staining with oxidized basic dyes is the chief concern. In water of sufficiently high oxygen content to prevent intracellular reduction of the dye, organisms or parts with little regional differentiation along the polar axis, coelenterate blastulae and planulae, echinoderm blastulae and gastrulae, the body of hydra, the naked stem of *Corymorpha*, hydroid tentacles, etc., stain uniformly or almost uniformly at first; that is, there is little or no evidence of differential penetration of dye in the ectoderm of such forms. With continued staining, however, an axial differential often appears. The depth of staining becomes greatest apically or anteriorly, decreasing basipetally or posteriorly, and cytolysis and death produced by the dye follow the same course. A study of staining gradients in relation to susceptibility gradients and to the gradient problem in general has been made by J. W. MacArthur (1921) with protozoa, hydra, planarians, other flatworms, and annelids as material. Like others before him, he finds the basic dyes much more effective, both as dyes and as toxic agents, than the acid dyes. Acid dyes in neutral solution do not stain and are rarely sufficiently toxic to kill. Increased acidity increases effectiveness of acid dyes. In a planarian which shows certain minor differences in relative susceptibility to basic and acid agents (pp. 112-113), MacArthur finds that basic dyes act like acid agents, and acid dyes, when effective at all, like basic agents. He also finds that the staining gradient shows the same axial relations as the later

⁶ Ranzi e Falkenheim, 1937; Pitotti, 1939.

death gradient in the dye and in other agents: his results are confirmed by many other observations on staining and toxic effects of various dyes.⁷

In his discussion of the literature of vital staining MacArthur calls attention to the apparent relation between rate of staining and physiological condition of different regions and concludes from the evidence of his own and other work that the axial staining gradient cannot be due to a differential permeability in any merely physical sense. The cell surface is living and concerned in the activity of the cell. The staining and toxic effect of the dye depend on its adsorption, precipitation, flocculation, or chemical combination on or with cell constituents. Its continued entrance and accumulation within the cell depend on its removal from the solution in the cell.⁸

Evidence from other work also indicates that graded differences in vital staining, particularly in the simpler animals and earlier developmental stages, are associated not merely with differences in permeability but with graded differences in physiological condition of the cells. Numerous observations on vital staining of protozoa, blastulae, planulae, young hydroids, stages of reconstitution of *Corymorpha* and other hydroids, turbellaria, and microdrilous oligochetes agree with MacArthur's data in showing that with sufficient staining axial staining gradients usually appear which parallel closely the death gradients observed with other physical and chemical agents. The gradients of injury, cytolysis, and death, with further continued staining, coincide with the staining gradient.

A point of some interest in connection with this differential staining is that, when staining has progressed to a certain stage, the gradient of susceptibility to cyanide disappears, all levels being equally susceptible. With further staining the susceptibility gradient undergoes reversal, that is, the regions which were originally most susceptible to cyanide become least susceptible after injury by the dye. This reversal of differential susceptibility to cyanide along an axis by deep staining has been observed

⁷ Protozoa, Child and Deviney, 1926; Hydra, Child and Hyman, 1919; embryonic, larval, and adult hydroids, Child, 1919d, 1926a; larval stages of the polyclad, *Stylochus*, Watanabe and Child, 1933, and other turbellaria.

⁸ Hypotheses concerning the manner in which vital staining occurs are not lacking. Since MacArthur's work is under discussion, it may be noted that he suggests an electrochemical hypothesis. The cation of the dye, which is the color ion, unites with an intracellular anion, which is a product of catabolism; if such anions are formed by dissociation of amphoteric proteins as acids, their production should be decreased by acidity and increased by alkalinity and by abundant oxygen, as seems to be true. According to McCutcheon and Lucke (1924), however, external alkalinity favors, internal alkalinity decreases, staining with basic dyes. They suggest that these dyes combine with some acid cell constituent.

with methylene blue and some other basic dyes in several algae, hydroid planulae and later stages, and other invertebrates (Child).

Methylene blue is known to increase respiration in tissues of higher animals and to protect against the action of cyanide.⁹ Whether the reversal of the susceptibility gradient to cyanide by dyes is due to increased respiration of the more deeply stained regions, which antagonizes the action of cyanide, or to decreased respiration resulting from injury by the dye, and consequently decreased susceptibility to cyanide, has not been determined.

Recently differences in vital staining of regions and blastomeres in certain eggs and embryos have been interpreted as evidences of differentiation. In the absence of information concerning technique, concentrations used, periods of staining, and toxic effects of the dyes used, it is perhaps questionable whether some of the differences described—for example, in cleavage stages—are not due to other factors than differentiation.¹⁰ Deep staining of one cell earlier than others about it may in some cases indicate only greater susceptibility and earlier injury by the dye, possibly in connection with certain stages of the division cycle. Some basic dyes are much more toxic than others; but, in general, intracellular accumulation of dye beyond a certain point results in injury to the protoplasm, and with further staining cytolysis and death occur. The graded differences in these effects along physiological axes of many organisms suggest quantitative, rather than specific, regional differences, but they do not exclude the possibility that specific differences are present without effect on dye action or that the staining characteristics of certain cells or organs may be associated with their differentiation. It appears beyond question, however, that use of a wide range of dye concentrations and staining periods is necessary as a basis for conclusions concerning the significance of differences in staining.

DIFFERENTIAL REDUCTION AND OXIDATION OF VITAL DYES

Intracellular reduction with change or loss of color of certain vital dyes occurs under anaerobic conditions or when oxygen tension of the medium falls below a certain point. The oxygen-level at which reduction of a par-

⁹ See, e.g., Eddy, 1931; Gerard, 1932, pp. 504-7; M. M. Brooks, 1932, 1935; Chen, Rose, and Clowes, 1933; Marsh, 1934; Solandt *et al.*, 1934; Chrisler, 1935; Bodine and Boell, 1937, and citations by these authors.

¹⁰ Ries, 1936, 1937; Ries and Schölzel, 1934; Ries and Gersch, 1936; Gersch and Ries, 1937.

ticular dye occurs differs in different organisms, and regional differences in time and rate of reduction in the individual are associated with differences in rate of oxidation. With gradual decrease of oxygen after staining, such differences are most clearly seen. In embryonic stages and even in adult individuals of many animal species these differences appear not as sharply defined and bounded areas but as reduction gradients, that is, gradients of change or loss of color in definite and characteristic relation to physiological axes of the whole, of organ systems, and of loci of developmental activity, such as buds. If injury by the dye has not occurred, return of color after reduction may be brought about by readmission of oxygen. With small organisms in a small amount of water reoxidation may occur almost instantaneously or within a few seconds, but a gradient of return of color is usually temporarily visible. If not injured by lack of oxygen or dye, the same material may serve for repeated reduction and reoxidation.

A number of the basic dyes which are available as oxidation-reduction indicators can be prepared as reduced, colorless leucobases with either a reducing agent, sodium hyposulphite, or its formaldehyde compound, ron-galite, or by catalytic reduction with molecular hydrogen by a platinum or palladium catalyzer.¹¹ The leucobases penetrate more readily than the oxidized dyes, and the color appears in the material when it is exposed to oxygen. Leucobases prepared with hyposulphite, however, are much more toxic than the oxidized dyes, even those which stain readily in oxidized form; consequently, great care is necessary in their use. In regions of the living material injured by them reduction is retarded or does not occur at all. Leucobases catalytically prepared are less toxic, but oxidize rapidly on exposure to oxygen and enter living material without oxidation only under anaerobic conditions.

With many organisms it is possible to distinguish definite and constant regional differences in rate of appearance of color after penetration of the leucobase and exposure to oxygen or with hyposulphite-leucobase, as it enters the cells in an oxygen-containing medium. In other words, the leucobase is more rapidly oxidized in some regions than in others; an oxidation gradient may appear temporarily. Regional differences in rate of intracellular oxidation of leucobase have been regarded as indicating difference in oxidase activity; but since the leucobases oxidize rapidly on exposure to oxygen without a catalyst, it may be questioned whether this reaction is always an adequate indicator of oxidase activity alone. But whether the dye enters the cell or organism as leucobase or in its usual form as

¹¹ See Fischer und Hartwig, 1937, and their citations.

oxidized dye, the most readily observed and most conclusive evidence of oxidation-reduction pattern is obtained by intracellular reduction of the oxidized dye. This can be made to occur rapidly or slowly as desired. The material can be brought at once, after staining, into anaerobic conditions, where reduction will take place rapidly or decrease in oxygen may be brought about gradually by the oxygen consumption of the material itself in a sealed container of small volume. It is necessary to make certain that injury from lack of oxygen or accumulation of carbon dioxide or other products of metabolism does not occur before reduction.

Suitable concentrations and periods of exposure to the dye must be determined by trial for the organism concerned, with both oxidized dye and leucobase. Too high concentration or too long exposure results in differential injury and retardation or absence of reduction of dye in the injured region; consequently, the reduction pattern becomes different from that of the uninjured individual. An axial reduction gradient may even be the reverse of the normal, because the region which normally reduces most rapidly is also most susceptible to injury by the dye and, when injured to a certain degree, though still living, reduces least rapidly. Examples are given in the following chapter.

If a true picture of oxidation-reduction pattern is to be obtained, it is also highly important, with gradual oxygen decrease by the living material, that equal oxygen distribution in the medium be maintained; otherwise differences in reduction which have no relation to physiological pattern may occur. For example, when two or more eggs or embryos lie close together without change of position after staining, reduction usually occurs first in the adjoining regions of each, because oxygen decreases more rapidly between them than elsewhere. A region of an embryo remaining continuously in contact with the bottom of the container is likely to reduce before others. Free-swimming organisms in a sealed container provide more or less completely for equal oxygen distribution; but if they tend to aggregate, unequal distribution usually results. An aggregation of *Paramecium* reduces much more rapidly after staining with methylene blue than isolated individuals swimming free in the same preparation, and at a certain oxygen-level an individual which has become colorless in the aggregation becomes blue at once on leaving it. With nonmotile forms—cleavage stages, for example—in a small sealed container equal distribution of oxygen may be maintained by frequent change in position of the preparation with respect to gravity.

It is not to be assumed that a dye-reduction pattern—for example, an

axial dye-reduction gradient—must always, and of necessity, coincide with the respiratory gradient of the same axis or that a respiratory gradient must always be distinguishable in an axis which shows a dye-reduction gradient. Dye reduction is regarded as chiefly dependent on intermediate oxidation-reduction reactions rather than on total oxygen use. On the other hand, when respiratory gradient and dye-reduction gradient do coincide, each aids in confirming the significance of the other.¹²

DIFFERENTIAL SUSCEPTIBILITY

Living protoplasms may be stimulated or depressed, accelerated or retarded, in activity or specifically altered in condition by external physical and chemical agents; and any of these effects, beyond a certain degree or threshold, becomes injurious or lethal. Agents acting within the organism may also affect a particular cell or cell group, or an organ or organ system, in all these ways. These effects are indicative of the sensitiveness or susceptibility of living protoplasm to factors in its environment. If life is a continuous dynamic equilibration, protoplasmic susceptibility signifies that this equilibration process can be altered in various ways or arrested by external factors. We do not doubt that, as far as the organism is concerned, differences in susceptibility of regions and organs, whether specific for certain agents or not, depend upon differences in physicochemical constitution and physiological condition or activity of the protoplasms, regions, or organs concerned. It is evident, however, that differences in susceptibility to a particular concentration or intensity of a particular agent tell us little or nothing more than that differences of some sort in constitution or condition exist. In order to obtain all the information possible concerning susceptibility to any agent, it is usually necessary to determine the susceptibilities of individuals, in different physiological condition and of different developmental stages, to a wide range of concentrations or intensities of the agent and often of other agents as well, to compare the susceptibilities of different species, and to check the data on susceptibility with those obtained by other methods. Conclusions concerning the nature of regional differences in protoplasmic constitution and condition have often been drawn from results obtained with a single agent in a single concentration or intensity. Such conclusions are often highly uncertain. Many external agents act more or less specifically on certain organs or organ systems of adult individuals, particularly of forms in which a high degree of histological differentiation is evident. A certain agent may act

¹² For further details concerning use of the method and for references, see Appendix II.

chiefly or perhaps entirely, so far as we can determine, on a certain organ; another on another organ. In case of some agents, action is more or less specific in a certain range of concentration or dosage; but above this range the action becomes more general, and in sufficiently high concentration or intensity such agents may affect the whole organism. For pharmacological purposes agents which produce physiological effects have sometimes been separated into those with specific and those with general action, but this distinction cannot usually be sharply drawn.

Another aspect of protoplasmic susceptibility appears in the relation between the susceptibility of a particular species-protoplasm, individual, or organ to a particular agent or to many agents and its physiological condition. For example, the physiologically young individual is usually more susceptible than the old to various agents within a certain range of concentration or intensity. Increase in motor activity increases the susceptibility of the planarian to cyanide in a certain range of concentration (Child, 1913a), but increased motor activity may decrease the susceptibility of many animals to alcohol and certain other agents within certain limits of concentration. Susceptibility of an organ system may change greatly during development. Susceptibility of the nervous system to anesthetics or to strychnine changes relatively to that of other organs from the medullary plate stage to the adult in vertebrates; but susceptibility of the hydroid nerve net to these agents undergoes no such relative change, and strychnine, like anesthetics, depresses nervous activity in the adult.

From the viewpoint of developmental physiology the questions of chief interest as regards susceptibility concern axial and regional differences in susceptibility, susceptibility patterns at the beginning and in early stages of development, and the changes which occur as development progresses. So far as data are available, axial or other regional differences in susceptibility which are specific for particular agents are usually less evident in earlier, than in later, developmental stages or absent, though regions of high yolk content in eggs may be highly susceptible to fat-soluble agents, and certain other differences which have been regarded as specific appear in some forms. Graded differences in susceptibility (susceptibility gradients) appear as characteristic features of early developmental pattern. As development progresses, these gradients may undergo alteration or even obliteration, and new gradients may appear; but in many of the simpler animals gradients of earlier stages persist throughout life. These gradients constitute what has been called "differential susceptibility." They are, in general, nonspecific to a high degree; that is, the gradient is



the same in direction with respect to developmental pattern, physiological axes, or radii for certain ranges of concentration or intensity of many different agents which act on living protoplasms in different ways.

Differential susceptibility to external agents appears in various aspects: differential death, differential inhibition, differential tolerance, differential acclimation, differential recovery, differential acceleration, and, in some cases in later stages, differential irritability and other functional differentials.

Using death or the cellular changes—cytolysis, coagulation, disintegration, etc.—associated with, or indicating, the approach of death, as a criterion, we find that death gradients are characteristic features of developmental pattern. For example, when the whole organism is equally exposed to action of an external agent in concentration or intensity above the limit or tolerance, but only slowly lethal, death occurs first in a certain region of a physiological axis and progresses along the axis from that region. In the earlier stages of buds and of organs which develop as budlike outgrowths from localized fields death progresses radially, though not always equally in all directions, from a region of highest susceptibility, which may be central or otherwise situated in the field. As such an axis develops, the radial susceptibility gradient becomes a longitudinal gradient. The differences in time of death at different levels of an axis may differ widely with different agents and with concentration or intensity of a single agent; but with certain ranges of concentration or intensity of many different agents, both chemical and physical, the progress of death is in the same direction.

Differential inhibition of embryonic or reconstitucional development or of other activities may occur with concentrations or intensities of many agents which are more or less toxic or inhibitory but not directly lethal. In consequence of differential inhibition developmental form and proportion can be modified in definite and controllable directions.

With a certain range of lower concentration or intensity, which, of course, must be determined experimentally for each species and each agent, and usually for different developmental stages and for physiologically young and old individuals, evidences of differential tolerance and of ability to acquire increased tolerance have been found in cases examined. In earlier publications this differential in degree or limit of tolerance or threshold of toxic or lethal action of the agent and the differential acquirement of increased tolerance have been grouped together and called “differential acclimation.” Now, however, it seems desirable to distinguish, at

least formally, two aspects of this differential: first, the differential in the threshold of irreversible toxic or lethal effect of the agent or the upper limit of tolerance to it; second, a differential in the rise of this threshold, that is, in acquirement of increased tolerance during continuous exposure to the agent. This is differential acclimation or conditioning in the strict sense. Apparently, there is in such cases a differential in the ability of the protoplasm to dispose of the agent in some way or to render it nontoxic or less toxic. The tolerance differential may be so great that a part of the individual finally dies after days or weeks of exposure to the agent, while the other part continues to live indefinitely; or, with effect of agent near the upper limit of tolerance, the whole individual may finally die, but the progress of death along the axis is in the reverse direction from that with action of the same agent above the limit of tolerance. *Dugesia* (= *Euplania*) serves as an excellent example. In concentrations of ethyl alcohol which kill within a few hours, physiologically young animals are more susceptible than old, and the head and posterior zooid region are most susceptible, death progressing posteriorly from the head in the anterior zooid. In a certain range of lower concentration young animals are more tolerant than old; and, even though both young and old are at first anesthetized more or less completely, the young recover activity before the old with continuous exposure to the alcohol, apparently because of acquirement of increased tolerance. Also, if death occurs anywhere, it begins at the posterior end of the anterior zooid and progresses anteriorly; it may stop at a certain level, and anterior region and posterior zooid region may continue to live indefinitely in the same concentration of alcohol; the anterior part may even develop a posterior end, and the posterior zooid a head, in the same concentration which was earlier partially lethal. Differential tolerance and apparently also some degree of differential acclimation or conditioning appear in early developmental stages with some agents in secondary differential modifications of form and proportion in directions opposite to those of the primary differential inhibition. In echinoderm development regions most inhibited or killed by more extreme toxic action show relatively more rapid development and larger size than others (chap. vi). These modifications occur even though all parts are more or less inhibited; and they follow a differential inhibition in earlier stages, indicating some degree of differential conditioning. Since the regions which are primarily most tolerant show the greatest capacity to acquire increased tolerance, the modifications of form and proportion resulting from differential conditioning are, in general, similar to those of differ-

ential tolerance. It is often difficult to determine whether the modifications observed are due merely to the primary differences in tolerance or to actual differential conditioning. Thus far, death gradients associated with differential tolerance and differential conditioning have been investigated in only a few forms and with a few agents, but difference in ability to tolerate and to acquire tolerance to different agents is evident. The differential in tolerance to ethyl alcohol along the polar axis of forms tested is considerable, and differential conditioning occurs relatively rapidly; but differential tolerance to cyanide is slight, and differential conditioning occurs very slowly if at all (Child, 1932*b*). It is possible that the mechanism of tolerance to certain agents may gradually fail with time and that a progressive differential sensitization to certain agents may conceivably occur, but evidence is lacking on these points.

Living organisms are able to recover more or less completely from less extreme toxic action of external agents after return to the normal medium. Axial differentials in recovery after slight differential inhibition are, in general, parallel to those of differential tolerance and differential acclimation but are often more strongly marked, since the inhibiting agent is no longer present in the recovery period. With more extreme action of the agent the more susceptible regions may be injured to such an extent that they cannot recover, even after return to normal environment, and only less susceptible regions recover. This is not, properly speaking, a differential recovery but rather a partial recovery, that is, a recovery of the least susceptible and least inhibited part; usually it is by no means complete and may be little more than a continuation of life after return to the natural medium. The parts that do not recover usually die, though in some cases they may remain alive in highly inhibited condition.

Differential tolerance, conditioning, and recovery are all secondary modifications, as far as distinguishable alterations of pattern are concerned. They depend on the physiological condition and metabolism of the part concerned, rather than directly on the action of the external agent. As regards both differential death and modification of form and proportion in development, their effects are the reverse of the primary inhibitions in their relations to physiological pattern.

As regards the physiological significance of differential susceptibility, the fact that the lethal gradient is the same in direction for many different agents, both chemical and physical, indicates that it does not depend on the constitution or nature of the agent. It is certain that lethal effects of different agents are not all brought about in the same way. The only con-

clusion possible seems to be that the susceptibility gradient represents a quantitative differential or gradient of some sort. In other words, differential susceptibility indicates quantitative features of physiological axes but gives no information concerning qualitative differences of substance which may or may not be present at different levels. Differential permeability may be a factor in determining differential susceptibility to some agents, but it is not the only or the chief factor, for the same gradients appear with agents that penetrate readily at all levels, with agents that penetrate only as they injure cell surfaces, and with physical agents, such as ultra-violet, X-rays and radium, temperature extremes, and lack of oxygen. Moreover, in all cases involving the same material, susceptibility gradients parallel very closely the respiratory gradients, gradients of dye reduction, and indophenol gradients. Differential tolerance and differential conditioning must involve a differential activity of the organism in relation to many agents. The anterior region of *Dugesia* is more tolerant and acclimates more rapidly or more completely to low concentrations of alcohol, and to some extent to low concentrations of cyanide, but is more susceptible to lethal concentrations than more posterior regions (Child, 1911c, 1913b, 1914b, 1932b). Also, physiologically young planarians are more tolerant to low concentrations, and more susceptible to high concentrations, than old. It has been suggested in earlier papers that a general parallelism exists between differential susceptibility and metabolic, respiratory, or oxidative rates at different levels of the individual organism. That this parallelism is absolutely complete or universal has not been maintained, nor need it be assumed that all the agents used in demonstrating differential susceptibility act directly on oxidation, reduction, or other metabolic reactions. However, it does appear highly probable that any sort of disturbance of the protoplasmic system by an external agent, if sufficient to bring about death or inhibition as direct effect, will be likely to kill or inhibit earlier in regions in which change is going on more rapidly than in those of less activity and that regions of more intense metabolism will have, in general, a higher tolerance and a greater ability to acclimate to, or to recover from, effects which are not too extreme. If a living protoplasm is a system undergoing continuous dynamic equilibration, any action of an external agent, if sufficient in amount or intensity, must sooner or later, directly or indirectly, alter some essential factor or factors of the system so that alteration of the whole system results, or its continued existence becomes impossible. The higher the rate of equilibration in a particular region of a physiological axis, the earlier will such alteration or destruction

occur, as in differential death and differential inhibition. On the other hand, if the external action is slight in amount or intensity, the more rapidly equilibrating region will eliminate in some way the disturbing factor or equilibrate to it more rapidly than a region which is equilibrating more slowly. This is apparently what happens in differential tolerance, differential conditioning, and differential recovery. For susceptibilities which are specific for particular agents, these relations do not hold; but they may hold for different regions of a part or organ which, as a whole, is specifically susceptible. Moreover, it is not necessary to assume that respiration or oxidation is always the primary factor in the various expressions of differential susceptibility, but it appears impossible to account for the facts independently of metabolic activity; and in most organisms respiration or oxidation-reduction appears to be a more or less trustworthy indicator of such activity, or at least of some of its fundamental factors. The relation between respiration or oxidation and susceptibility may not be the same in all organisms or in all organs of adult individuals; when glycolysis is the chief source of energy, it is obviously different from that in aerobic respiration. All that is maintained here is that there appears to be a general parallelism between the phenomena of differential susceptibility, particularly in early developmental stages and in the simpler organisms, and the basal metabolic activity of the protoplasmic system concerned. Differential susceptibility is at best merely one method of indicating differences in physiological condition which appear to be quantitative and which, as will appear in following chapters, are essential factors in development; without the aid of other methods it gives no direct information as to the nature of the differences indicated.¹³

THE QUESTION OF DIFFERENTIAL PERMEABILITY

It was pointed out above that gradients indicated by external chemical agents are not dependent merely on a permeability gradient. Nevertheless, the question whether a differential permeability is present along physiological axes as one feature of the axial gradient is of interest. It might perhaps be expected that rate of staining by vital dyes would throw some light on this question. MacArthur (1921) maintained that differential permeability alone could not account for the gradients of staining and susceptibility to the dyes. The writer's observations bear out his conclu-

¹³ For references to the literature on differential susceptibility and further discussion of the question of its relation to metabolism see Appendix III.

sions. With low concentrations of methylene blue the entoplasm of *Paramecium* stains before the ectoplasm, and the posterior before the anterior ectoplasm. With a certain higher range of concentrations ectoplasm and entoplasm stain about equally. With still higher concentrations ectoplasm stains before entoplasm, and anterior ectoplasm finally becomes more deeply stained than posterior (Child, 1934*b*). Other protozoa show similar relations, and in metazoa staining gradients in opposite directions along an axis have been observed with low and high concentrations of dye. Even when earlier stages of staining are quite uniform along an axis, as far as can be determined, a very distinct gradient in depth of staining and in toxic or lethal effect appears later. This susceptibility gradient evidently results from difference in condition inside the cells and a consequent difference in adsorption or combination of the dye, rather than from any difference in permeability. Experiments with *Paramecium* on penetration of ammonia and acetic acid and with hydroids, using neutral red as indicator, have shown some evidences of an axial differential permeability to extremely low concentrations.¹⁴ These concentrations, however, are only slightly or not appreciably toxic; and with the higher concentrations, in which a lethal gradient appears, penetration seems to occur equally at all levels. Strong bases and strong acids do not alter the color of the intracellular neutral red until cytolysis begins, but the death gradient is the same as with weak bases and acids. A very distinct susceptibility gradient appears in single elongated cells of monosiphonous algae, even though no difference in penetration or depth of staining can be observed (Child, 1916*c*, *e*, 1917*a*, 1919*f*).

ELECTRIC POTENTIAL DIFFERENCES

For many years electric potential differences and their changes with activity have been investigated in nerve and muscle, and numerous observations have been made on other organs of plants and animals. Most of these investigations, however, have been on organs of fully developed individuals, and the work on nerve and muscle has been concerned very largely with vertebrate material. These investigations, important as they are, are only remotely connected with problems of developmental pattern. Potential difference in relation to developmental pattern and physiological axes has received less attention, but data on axial potentials in certain

¹⁴ Child and Deviney, 1926; Child, 1926*a*, and unpublished data.

plants and animals and their changes under experimental conditions have been obtained, though largely with growing plants and adult animals.¹⁵

It is evident from the data that potential gradients are characteristic features of physiological axes, but different authors are not in complete agreement as regards direction of change in sign. Mathews; Morgan and Dimon; Hyman; Hyman and Bellamy; and Watanabe found in various invertebrates a decrease galvanometrically in negativity from the apical or anterior region basipetally or posteriorly and in annelids a second gradient of negativity decreasing from the posterior end anteriorly to a certain level. According to Lund and his co-workers, galvanometric positivity decreases from the apical region basipetally and increases again toward the basal region of the hydroid *Obelia*. Also, the tips of stem axes of the Douglas fir are galvanometrically positive (internally negative) to lower levels, and in the onion root there is a decrease in galvanometric positivity from the tip basipetally to a certain level with some increase farther basally. Barth, working with hydroids, does not find any constancy in direction of potential difference. In *Tubularia* the hydranth is negative to the middle stem region, and a cut or reconstituting end is usually negative to a hydranth. In *Eudendrium* reconstituting regions are usually positive to other regions. In this connection it may be noted that hydranth reconstitution in *Tubularia* is a redifferentiation of a portion of the stem without outgrowth, while in *Eudendrium* and *Obelia* outgrowth of tissue from the cut end occurs before the hydranth develops.

As regards hypotheses concerning the origin of bioelectric potentials, Du Bois Reymond held that they originated in purely physical factors; among the earlier physiologists, Hermann, Hering, Biedermann, and Waller did not accept this view but maintained that they were associated in some way with reactions of metabolism, though Hermann apparently discarded this hypothesis later. In general, the hypothesis of a relation to metabolism seems to have been regarded more favorably than others.

¹⁵ Numerous determinations have been made on plant axes, usually showing the tip electronegative to lower levels, a few with the reverse potential difference. The following references are more or less directly concerned with axial potentials in animals: Mathews, 1903, hydroids; Hyde, 1904, eggs of fish and turtle; Morgan and Dimon, 1904, earthworm; Hyman, 1918, 1932b; Hyman and Bellamy, 1922, sponges, hydroids, hydromedusae, planarian, annelids; Child and Hyman, 1926, hydroid; Lund, 1921c, 1922, 1923a, 1924a, b, 1930, 1931a, b, c, 1932a, b; Lund and Kenyon, 1927; Lund and Bush, 1930; Lund and Hanszen, 1931; Lund and Moorman, 1931; Marsh, 1928, 1930, 1932; Rosene, 1930; Rosene and Lund, 1934, hydroids, plant stems, and roots; Watanabe, 1928, earthworm; Burr, 1932; Burr and Lane, 1935; Burr and Hovland, 1937a, b, developmental stages of amphibia and mouse; Barth, 1934b, hydroids; Iwasama, 1935, artificially fertilized amphibian egg.

Hyman (1918), Hyman and Bellamy (1922), and Child and Hyman (1926) suggested that the axial potential difference observed in many animals originates in metabolic differences. Lund and his co-workers, in various papers from 1926 on, have maintained, as the result of determinations of oxygen consumption and comparative estimations of CO_2 production, decrease of potential difference and of oxygen consumption by cyanide, differential reduction of methylene blue, and other experiments, that electric polarity is quantitatively correlated with oxidation—in other words, that the axial potential differences are expressions of an axial metabolic gradient. It was further suggested by Child (1924*b*, chap. xi) that, if the potential differences give rise to currents under natural conditions, such currents may be important factors in physiological dominance and correlation. In his later papers Lund assumes that currents do flow under natural, as well as under experimental, conditions, and emphasizes their importance as factors in correlation. Barth, on the other hand, finds “no coincidence of electric polarity and organic polarity in different hydroids”; that is, in some forms the apical region is galvanometrically positive, in others negative, to lower levels, and in isolated pieces cut ends may be at first negative, later positive, or continuously positive to other levels, according to the species. In view of the fact that potential difference appears to be a characteristic property of physiological axes, it seems rather improbable that no definite relation between it and other axial characteristics exists. Moreover, comparison of the apical regions and of the manner in which hydranths are reconstituted in the hydroid species in which potential differences have been determined suggests that apical galvanometric negativity may be associated with activity of differentiated or differentiating hydranths or with the stimulation following section and apical positivity with predominance of growth activity. Again, assuming that current results from the potential differences, the current may have different effects on regions which it reaches, according to the sign of the apical region. A current resulting from galvanometric negativity of the apical region or cut end may conceivably result in activation and dedifferentiation of cells which it reaches and organization of a hydranth primordium from a part of the stem without outgrowth, as in *Tubularia* (see Fig. 13); and current from an electropositive end may establish a growth gradient, such as appears in *Obelia*. According to Barth (1934*a*), inhibition of reconstitution by an applied current may occur at either cathode or anode according to species; and in *Tubularia*, according to current density. It is to be expected that reconstitution will be inhibited, either by inhibition of the ac-

tivation following section, which is apparently the primary factor in determining the development which follows, or by inhibition of the resulting growth or differentiation, or by both. The results as regards cathodal and anodal inhibition in different species and with different current densities may perhaps mean that, in the one case, one of these activities, in the other case, the other, is inhibited. But aside from the question of interpretation, an electric-potential gradient is certainly characteristic of the polar axis of plants and animals, as far as investigated, parallels closely other gradient expressions, and appears to be correlated with metabolic activity; but many questions and problems await further investigation.

In many animals galvanotactic reactions show a definite relation to the polar gradient. The reaction, however, cannot be regarded as a simple attraction of unlike poles resulting from an electric gradient in the organism but is a physiological reaction to an external differential and can be altered or reversed by change in environment or physiological condition.¹⁶

OTHER METHODS CONCERNED WITH PATTERN

Other biophysical, biochemical, and histochemical methods give evidence of differential distribution or regional localization of substances in relation to developmental pattern. As differentiation progresses, many such localized differences of course appear as consequences of the earlier pattern; but some quantitative differentials, in content of water, fat, carbohydrate, etc., may persist or appear in relation to axiate pattern in adult individuals. The nitroprusside reaction indicates a polar glutathione gradient in some forms; in others no differential has been found. A modification of the nitroprusside reaction has been used by J. Brachet (1938) as an indicator of differential distribution of SH-proteins in amphibian development.

Evidence concerning early developmental stages bears more directly on the problem of pattern. A differential localization of sulphydryl proteins in very definite relation to developmental pattern in the amphibian egg and embryo has recently been reported (J. Brachet, 1938). By staining and injecting eggs with acid-alkali indicators the appearance of a polar differential in hydrogen-ion concentration at the time of polar-body formation has been observed in eggs of certain annelids, mollusks, and teleosts, resulting finally in some eggs in sharply defined acid and alkaline zones.¹⁷ The question of distribution and disappearance of glycogen has

¹⁶ Hyman and Bellamy, 1922; A. R. Moore, 1923; Hyman, 1932*b*.

¹⁷ Spek, 1930, 1933, 1934*a*, *b*, *c*.

arisen in connection with amphibian gastrulation and induction (see pp. 154, 477). Presence of axial functional differentials has been demonstrated or indicated in many organisms and organs in many ways. Functional dominance of apical or anterior regions is a familiar fact. In planarians and hydroids any body-level dominates, to some degree, more basal or more posterior levels, provided more apical or anterior regions are absent. The central nervous system in general, the ctenophore plate row and its conducting path, the heart, the mammalian alimentary tract, and particularly the small intestine, all present somewhat similar relations of dominance.

Study of the physical condition of protoplasm may show regional differences or chronological changes in viscosity, gelation, etc., which are related in some way to developmental pattern. Certainly the colloidal differences between the cell surface and interior are fundamental factors in the pattern of the cell as an organism. With the aid of polarized light and X-rays much has been learned concerning the ultra-structure of many morphological differentiations and products of living protoplasms, muscular and other fibers, both permanent and temporary, membranes, chromosomes, cellulose, hair, etc. The crystalline or other orientations of molecules or micellae discovered or inferred appear, in general, to be characteristic of rather highly differentiated protoplasms or products or related to local conditions, mechanical tensions or pressures, surfaces, or interfaces. Up to the present no fundamental ultra-structure of a protoplasm that might be regarded as the foundation of organismic and developmental pattern has come to light. Many of these ultrastructural patterns are more or less continuously forming and disappearing in protoplasms, and those that are persistent and associated with morphological differentiation are apparently derivatives rather than fundamental factors of pattern.

METHODS OF ANALYSIS OF DEVELOPMENTAL POTENTIALITIES AND POTENCIES

As the words are used here and in following pages, "potentiality" means possibility and "potency" means power or ability. Not all developmental potentialities are realized in development under natural conditions, for realization of all potentialities development in all possible environments is essential; it is impossible to determine what the potentialities are in any other way. Developmental potency, the ability to develop in a certain way, represents the potentiality which is realized in a particular environment, intraorganismic or external. In the course of develop-

ment potentialities may become progressively more limited, at least as far as can be inferred from behavior of the material in certain environments; but we cannot be certain that in some other environment the potentialities supposedly lost might not be realized. New potencies, however, may appear as development progresses: the ability to develop in a certain manner is attained only when a certain stage is reached, that is, when the developing system has attained a certain physiological condition in consequence of changes occurring within itself, or in its intra- or extra-organismic environment, or both. In case of a developing part of the intact organism, both the part and its intraorganismic environment are apparently factors in determining its developmental potency or potencies (cf. Gilchrist, 1937*a, b*).

The methods most widely used in attempts to analyze potentialities and potencies are those that are not infrequently regarded as the methods of developmental physiology—isolation, explantation and transplantation or grafting of parts of organisms, union of individuals, and isolation and aggregation of cells. The method of differentially modifying development by exposure of the whole developing organism or system to experimental environments, which was mentioned above under differential susceptibility, also serves for the realization of other developmental potentialities than those realized under natural conditions.

ISOLATION OF PARTS

Isolation of parts in experiment has usually meant physical isolation. The part is separated from other parts and remains in the normal external medium or may be brought into an experimental environment. An isolation experiment involves at least two, often more than two, parts. A hydroid or a planarian may be separated into two or many parts; the blastomeres of a two-cell or later stage may be isolated; a small part, an organ primordium, a limb bud, the optic primordium, etc., may be isolated from other parts, but those parts are also isolated from it, and experiment may be concerned with the effect on either or both.

Physiological isolation of parts without physical separation occurs in many organisms in connection with agamic reproduction and with function and probably to a greater or less extent in development of the individual (see chap. ix). Results of physiological isolation are, in general, essentially similar to those of physical isolation as regards further development, though they may be less extreme.

EXPLANTATION

As the term is usually employed, explantation is merely isolation of a part in a special extraorganismic environment. This environment may be supposedly indifferent: a balanced salt solution such as the various concentrations and modifications of Ringer solution adjusted to the organisms concerned. If properly adjusted, such a medium prevents the loss of electrolytes which may occur in water, particularly if isolation involves a wound. Cultures *in vitro* of living cells, cell masses, organ primordia or organs in special media, plasmas, and gels (nutritive, growth-stimulating, etc.) are explantations. These experiments involve not only the effect of isolation but also that of the medium on the isolated part. Explantation methods have been extensively used in the culture of particular cells or tissues of embryonic or later stages, but recently culture *in vitro* of larger parts of embryos, chiefly of vertebrates, has been undertaken in the attempt to provide environments favorable to further development.

TRANSPLANTATION OR GRAFTING

Transplantation consists essentially in altering the organismic environment of the part concerned. This can be accomplished in many ways: transplantation may be autoplasmic, to the same individual, homoplasmic, to another individual of the same species, heteroplasmic, to another species of the same genus, xenoplasmic, to an individual of another genus, family, order, etc. In any of these relations it may be orthotopic, to the position originally occupied by the part concerned, or heterotopic, to some other position. The part may be transplanted in normal orientation to the axiate pattern of the host or in some other orientation. Donor, transplant, or host may be subjected to experimental conditions before or after transplantation. Size of transplant and developmental stage of donor or host can be varied. Transplantation to the extraembryonic membranes of birds is possible; this environment is still physiological but more or less isolated from the axiate pattern of the host. Up to the present the most extensive transplantation experiments with embryonic stages have been made with amphibian and avian material, but transplantations of parts of fully differentiated hydroids, planarians, and annelids have given results of great value for analysis of developmental pattern.

FUSION OF INDIVIDUALS

With some organisms it has been possible to unite developmental stages of two intact individuals. Here the questions are whether they can recon-

stitute to a single individual or retain their individuality and whether one may dominate the other and alter its development. The question whether fusion of individuals of different races or species is possible also arises.

DISSOCIATION AND AGGREGATION OF CELLS

Cells of certain sponges and hydroids can be dissociated and, in contact with each other, may aggregate into masses, which may be varied in size as desired. Under certain conditions these masses develop into complete individuals. These cases raise questions of the origin of developmental pattern and of the possibility of cell dedifferentiation and redifferentiation in relation to the new pattern.

GENERAL PURPOSES OF THESE METHODS

These methods of experiment have been developed in the attempt to obtain information concerning developmental potentialities and potencies. By means of them we endeavor to discover whether, or to what extent, realization of potentialities or developmental expression of potencies is intrinsic in the part concerned or is dependent either on a particular regional relation to other parts of the organism or on general factors of intraorganismic environment, and whether or how it is affected by different nonorganismic environments. Analysis of physiological dominance, both in preventing physiological isolation and in determining the course of development of subordinate parts (induction), has been greatly advanced by these methods.

When isolated or transplanted parts undergo reconstitution, that is, alteration of pattern, we infer that they or some of their cells are not so stably differentiated or their course of development so unalterably established that they cannot react to the altered conditions, and that their developmental or other behavior as parts of the intact individual must be dependent, at least in part, on factors in their intraorganismic environment. If they continue to develop or otherwise behave as in the intact individual, intrinsic factors independent of other parts are supposedly concerned. As regards development, these differences are commonly distinguished, following Roux (1885), as dependent or correlative differentiation and independent or self-differentiation. Actually, however, the difference involves not only differentiation but the whole pattern of developmental and other behavior. A part may be independent in certain respects, dependent in others. Moreover, as Roux pointed out, a development which is independent as regards relation to other parts is dependent on relations

within its developing system. As far as relations to other parts are concerned, a reconstituting hydroid or planarian piece is undergoing self-development; but within the piece reconstitution depends on an orderly and definite pattern of relations, on dominance of certain parts and subordination of others. Self-differentiation or self-development occurs only when a pattern already present persists in the part after its isolation or transplantation. Such a part is said to be "determined," that is, its pattern is regarded as fixed. Labile and definitive determination are often distinguished. It cannot be too strongly emphasized, however, that determination is always relative to a particular environment or environments. To conclude that a pattern is unalterable because it is not altered by isolation or by transplantation is entirely unjustified. There is always the possibility that in some other environment it may undergo alteration. In fact, this occurs in some transplantation experiments; the pattern of a part transplanted to a certain region of a host may persist, but with transplantation to another region it may undergo alteration. Undoubtedly, progressive stabilization or fixation of pattern or the basis of differentiation does occur in the course of development, even before pattern or differentiation become visible. Determination, in the sense of a more or less stable disposition or tendency to develop in a certain way before that development becomes evident, obviously represents a change from an earlier undetermined condition. Isolation or transplantation may give evidence of it but tells us nothing concerning its nature. To say that a part is determined means only that it possesses a certain disposition as regards further activities and involves no implications concerning the nature of that disposition. If we keep in mind the possibility that even the most obstinate dispositions may not be unalterable and that the word "determination" is an expression of our ignorance, it serves a useful purpose.

CHAPTER IV

PHYSIOLOGICAL CHARACTERISTICS OF AXIATE PATTERNS

BEFORE turning to the experimental analysis of morphogenesis, "determination," and differentiation, it seems desirable to devote some attention to certain physiological characteristics of axiate pattern. Since agamic development and reconstitution of isolated cells or pieces occur in many organisms in a definite relation to the pattern of the parent, some of the physiological features of adult pattern, as well as those of early developmental stages, are of interest in this connection.

PLANTS

A few points concerning plants are noted because they suggest certain similarities of plants and animals as regards general pattern. In some fifty species of axiate algae examined with various agents, differential susceptibility decreases basipetally in axes with apical growing tips, at least in the younger parts of the axis (Child, 1916*c*, *e*; 1917*a*, *b*; 1919*f*). In the older parts of the thallus irregularities often appear. Multiaxiate forms with regularly arranged bipinnate or radial branches show the basipetal gradient in each branch and in the system as a whole; that is, the susceptibility of the growing tips of branches decreases from branch to branch basipetally. In axes consisting of a single series of elongated cells the progress of cytolysis can be observed, not only from cell to cell, but along each cell from the distal to the proximal end. The whole bipinnate thallus of the alga, *Bryopsis*, is a single cell; but the gradient is the same as in similar multicellular axes (Child, 1916*e*). In axes with a basal growing region susceptibility decreases from the base acropetally; the frond of the kelp *Nereocystis* (Child, 1919*f*) and hairs of the *Fucus* thallus (Child, 1917*a*) are examples. Unpublished data on the differential susceptibility of *Volvox* to a large number of agents (see Appendix III, p. 735) show decrease from the pole of the growing region, the posterior pole in locomotion.

Polarity of the egg of the alga *Fucus* can be determined by various external differentials (see pp. 423-25). Light is probably the usual determining factor under natural conditions, but there is evidence that the egg

has a polarity at the time of discharge from the thallus that may be effective in the absence of sufficiently intense external differential after discharge. The rhizoid outgrowth appears on one side of the cell before the first division, and the plane of the first division is normally at right angles

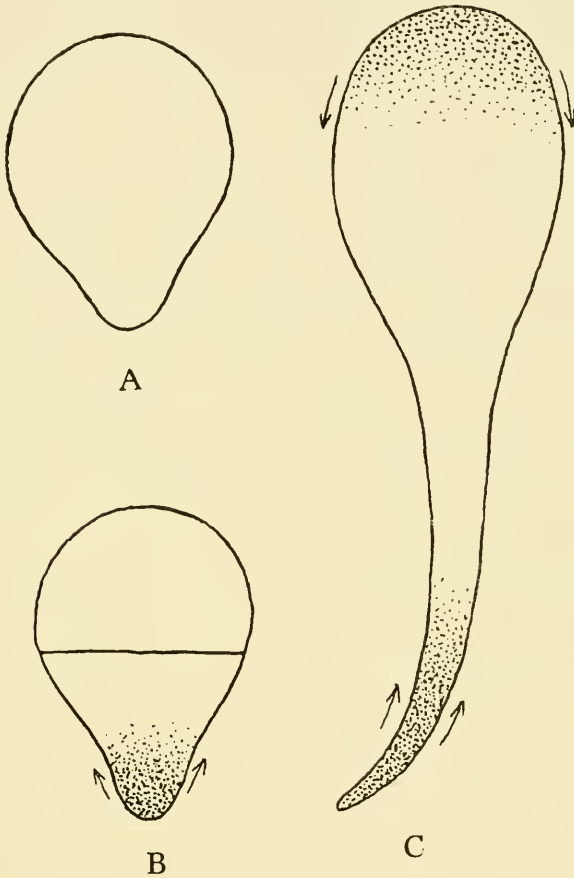


FIG. 26, A-C.—Three developmental stages of the alga, *Fucus*. A, growth of rhizoid preceding first division; B, two-cell stage, rhizoid gradient; C, later stage, thallus and rhizoid gradients.

to the direction of rhizoid outgrowth (see Fig. 26, A, B). The pole opposite the primary rhizoid becomes the apical pole of the thallus. No definite death gradient has been observed in the eggs before determination of polarity by light or some other external factor; but when the rhizoid begins to grow out, it becomes a region of high susceptibility, and death pro-

gresses basipetally from its tip (Fig. 26, *A, B*). Only in somewhat later stages, when the developing thallus consists of several or many cells, does its apical region become more susceptible than other parts. From these stages on, cytolysis progresses basipetally from the tip of the thallus and from the tip of each rhizoid present (Fig. 26, *C*). Reduction of potassium permanganate shows the same gradient pattern (Child, 1919*a*). An electric polarity is present in single cells of *Pithophora* and *Nitella* (Lund, 1938). Mold hyphae developing from spores show a basipetal susceptibility gradient.

Axial differences in rate of respiration in the higher plants are often of uncertain significance because the proportions of active and relatively inactive or dead cells and of protoplasm and water, cellulose or other non-living substances differ at different levels. Structural differences along the axis of the potato tuber are not great before sprouting occurs: basal halves produce from 6.5 to 6.8 per cent less carbon dioxide per unit of weight than apical halves in a variety tested; after apical sprouts begin to grow, the difference is 41.8 per cent, and after removal of sprouts, 10.7 to 19.8 per cent (Appleman, 1915). The fact that apical "eyes" tend to develop first and inhibit others more or less completely suggests that there is an axiate pattern in the quiescent tuber and that the difference in CO₂ production is a feature of it.

Many observations by many investigators have shown that electric polarity is a characteristic feature of plant axes, and various sources of origin of the potential differences observed have been suggested. According to Lund and his students, the growing tip of the onion root and of other roots and the tips of the main stem and branches of the Douglas fir are externally electropositive to lower levels of the same axis, and the tip of the main axis of the fir is positive to the tips of lateral branches of the first whorl.¹ Rate of methylene blue reduction and CO₂ production in the root are highest in the most positive region and decrease basipetally with it to a level some distance from the tip, where there is a region showing some increase, followed again by decrease. In both root and fir stimulation of a tip decreases its positivity and may reverse the potential gradient. The electric polarity of the fir shows a differential susceptibility to temperature. Decrease in temperature decreases electric polarity because decrease of positivity is greater in regions of high than of low positivity; in other words, a differential inhibition apparently occurs. With return to the

¹ Lund, 1928*a, b, c*, 1929*a, b*, 1930, 1931*a, b, c*, 1932*a, b*; Lund and Kenyon, 1927; Marsh, 1928.

original temperature the most highly positive region shows a greater "rebound" than lower levels (differential recovery).

The root tip shows an essentially similar relation between potential difference and local deprivation of oxygen. Removal of oxygen from about regions of the root which have oppositely oriented electric polarities produces opposite effects on the potential of the whole. Removal of oxygen from the region of active cell division at the root tip greatly decreases and often reverses the potential difference between this and a lower root-level, that is, the effect is differential (Rosene, 1934; Rosene and Lund, 1935). Presence of a glutathione or SH-gradient in the growing tips of certain plants has been reported (Camp, 1929); this perhaps indicates a gradient in oxidative metabolism. Lund holds that the axial electric-potential gradient results from a gradient in rate of oxidation.

Determination of oxygen uptake and of flocculability of colloids in parts of flowers show parallel respiratory and flocculation gradients corresponding to the morphological symmetry of the flower concerned. Results with bilateral flowers are particularly interesting: the gradients are in the direction of the plane of symmetry. In leguminous flowers tested, rate of respiration decreases and flocculability of colloids increases from the superior to the inferior parts of the corolla; in some other bilateral flowers the gradient is in the reverse direction (Zanoni, 1934*a*, *b*).

Analysis shows gradients of various substances along the axes of the higher plants. These, or some of them, are merely indicative of progressive changes in physiological condition and progressive differentiation with increasing distance from the growing tip. They are consequences of the type of pattern characteristic of most plant axes, a growing tip remaining embryonic and continuously giving rise to new cells, all or a part of which gradually differentiate. In general, it seems evident that physiological gradients are characteristic and essential features of axiate pattern of plants. Their presence can even be shown in single elongated cells of some multicellular axes, and they are present in multiaxiate unicellular plants, such as *Bryopsis*. The axes of the higher plants are obviously gradients, at least in the younger regions near the growing tips. When isolated parts of a plant axis undergo reconstitution, these gradients play a part in determining localization of shoots and roots. Doubtless, gradients in roots and shoots and in other plant organs differ in character, but perhaps the presence of a gradient is just as significant in relation to the problem of pattern as the character of the reactions which occur in it and the chemical constitution of the substances involved.

PROTOZOA

An ectoplasmic gradient has been demonstrated in many protozoa, chiefly ciliates, by differential reduction of methylene blue and differential susceptibility to many chemical and physical agents, and the indophenol blue reaction and reduction of permanganate have been used with a few species. Results obtained with the different methods show a rather remarkable agreement.

In *Amoeba* susceptibility to cyanide decreases from tip to base of the pseudopod (Hyman, 1917); and, according to Lynch (1919), animals in the limax condition, that is, with a temporary anteroposterior axis, show an anteroposterior gradient in the whole body. Bovie and Barr (1924) have observed a similar gradient in susceptibility to radiation. Susceptibility and permanganate reduction decrease from the mouth region basipetally in *Noctiluca*. Evidence of a longitudinal ectoplasmic gradient has been obtained from all ciliates examined for the purpose—some thirty species; but *Paramecium* has been more extensively studied than other forms. With staining which is not appreciably toxic rate of reduction of methylene blue in *Paramecium* ectoplasm decreases from the anterior end posteriorly (Child, 1934*b*). In very low concentrations of oxidized dye the ectoplasm of *P. caudatum* does not stain appreciably, but the entoplasm stains slowly, at least in the posterior region (Fig. 27, *A*). In slightly higher concentrations anterior ectoplasm does not stain, while the posterior ectoplasm stains gradually and sooner or later shows toxic effect with loss of structure, beginning at the posterior end and progressing anteriorly, and finally cytolysis progressing in the same direction (Fig. 27, *B*). These differential stainings occur when the dye solution is exposed to air. They are not due to deeper staining of the posterior region in consequence of entrance of dye through the mouth into the posterior entoplasm and passage into the ectoplasm; the entoplasm may be uniformly stained throughout, with ectoplasmic staining only in the posterior region. With further increase of concentration, ectoplasm stains equally throughout; and with decrease of oxygen, rate of dye reduction decreases from anterior to posterior end (Fig. 27, *C*). Ectoplasmic reduction seems to occur a little more rapidly about the oral region than in the general ectoplasm of that body-level; this, however, is not entirely certain and is not indicated in the figures.

The facts suggest that some dye reduction occurs in the ectoplasm at oxygen-levels in media exposed to air. If this is the case, it appears that

with extremely low concentrations all levels of the ectoplasm may reduce the dye as rapidly as it enters; with slightly higher concentrations, the reduction gradient appears with rate of reduction decreasing from anterior to posterior levels. The only alternative to this suggestion is apparently a differential ability of the ectoplasm to decolorize the dye in some other way than by reduction.

In still higher dye concentrations the anterior ectoplasm becomes more deeply stained than other parts, and reduction is retarded in it or does not

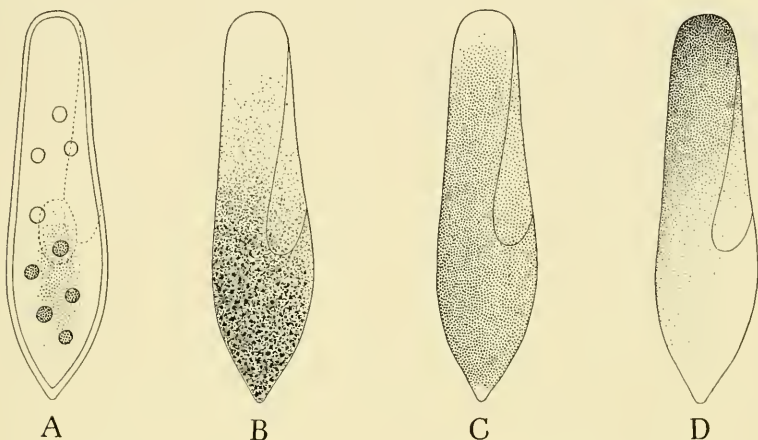


FIG. 27, A-D.—Differential staining and differential reduction in *Paramecium* (methylene blue). A, very low concentration, staining deepest parts of posterior ectoplasm first; B, higher concentration, stains posterior ectoplasm and with continued staining, injury, loss of structure and ability to reduce occur, progressing anteriorly while anterior region remains unstained; C, early stage of differential reduction after staining of whole ectoplasm; reduction progresses from anterior end posteriorly; D, high concentration or long staining in high oxygen; anterior ectoplasm stains more deeply than rest, and its reducing power is decreased or destroyed, while posterior region still reduces (from Child, 1934b).

occur, while more posterior regions are still uninjured and able to reduce (Fig. 27, D). When the leucobase of methylene blue prepared with hyposulphite (see p. 68) is added to culture fluid containing *Paramecium* and exposed to air, it penetrates at once and is almost immediately oxidized in the animals. For a few seconds, however, an oxidation gradient appears, rate of dye oxidation decreasing in the ectoplasm from anterior to posterior levels; but staining very soon becomes uniform. Extremely high dye concentrations, particularly of the leucobase with hyposulphite, are highly irritating and induce long-continued backward locomotion. Under these conditions the posterior ectoplasm oxidizes the leucobase and stains more

rapidly and more deeply than other regions, and cytolysis begins posteriorly, often in 1 or 2 minutes.²

Most of the observations on oxidation and reduction of dyes have been made with *P. caudatum*. The dye-reduction gradient is even more distinct in *P. multimicronucleatum*, decreasing posteriorly in uninjured animals and reversed in direction when differential injury retards or abolishes reduction anteriorly. As might be expected, the continuously circulating ectoplasm of *Paramecium* shows no evidence of an intrinsic gradient, the course of reduction in it being apparently determined by the ectoplasmic gradient. The indophenol blue reaction (see p. 64) with highly dilute reagents, so that the reaction occurs before the animals are killed, is most sharply localized in the inner portion of the ectoplasm or on the boundary between ectoplasm and entoplasm and decreases in rate from anterior to posterior end (Child and Deviney, 1926).

Differential susceptibility of *P. caudatum* to various gradually lethal chemical and physical agents, as indicated by cytolysis or other changes in the ectoplasm, decreases from the anterior end posteriorly, with a sec-

² Only the reversed reduction gradient resulting from differential injury of the ectoplasm by the leucobase, with retardation or absence of reduction anteriorly, was observed by Roskin and Semenoff (1933) with the use of a leucobase which was toxic, according to their own statement. Their conclusion that reduction occurs more rapidly in the posterior region is therefore mistaken. Using thionine reduced by rongalite (see p. 68) and oxidized Janus green, Gersch (1937) concludes that oxidation of the leucobase occurs more rapidly anteriorly than posteriorly in *Paramecium* and that reduction of the oxidized dye is more rapid posteriorly than anteriorly. No data concerning concentrations used or staining periods are given, but the rongalite-leucobase solution is stated to be somewhat toxic. The author concludes that oxidation is more rapid anteriorly; reduction more rapid posteriorly. The question how oxidation occurs in the anterior region without reduction or more rapid oxidation with less rapid reduction, and in the posterior region more rapid reduction with less rapid oxidation, is of interest in connection with this conclusion. Apparently the dye-reduction gradient decreasing anteriorly, as observed by Gersch, is, like that found by Roskin and Semenoff, the result of a differential toxic effect decreasing from the anterior end posteriorly, with reduction most retarded or absent anteriorly. (See also Kalmus, 1928.) Both the rongalite-leucobase and Janus green are toxic, the latter extremely so. That it is essential to use a wide range of concentrations of dye and, with oxidized dyes, different exposure periods, in order to avoid, as far as possible, misleading results, is sufficiently evident from the results obtained with *Paramecium*. It is possible to obtain both an oxidation and a reduction gradient in either direction, according to concentration of leucobase or oxidized dye used and according to staining period with oxidized dye. Only by varying the procedure sufficiently to determine which gradients most nearly represent physiological condition and which are results of differential toxic effect is it possible to attain an adequate basis for any conclusions. To assume that the animals are uninjured because they continue to swim is not justified. The anterior ectoplasm may be so injured that it is entirely unable to reduce the dye at all, while the animal is still able to swim. These reversed reduction gradients in *Paramecium* resulting from differential toxic effect of the dye are excellent examples of the differential susceptibility along the anteroposterior axis.

ond region of higher susceptibility at the extreme posterior end in some, not in all, individuals, perhaps associated with reconstitution after fission or possibly with backward locomotion.³ A characteristic effect of exposure to ultra-violet radiation is shortening of the anterior region (Fig. 28, *A-C*); with sufficient exposure this may be followed by complete cytolysis of the

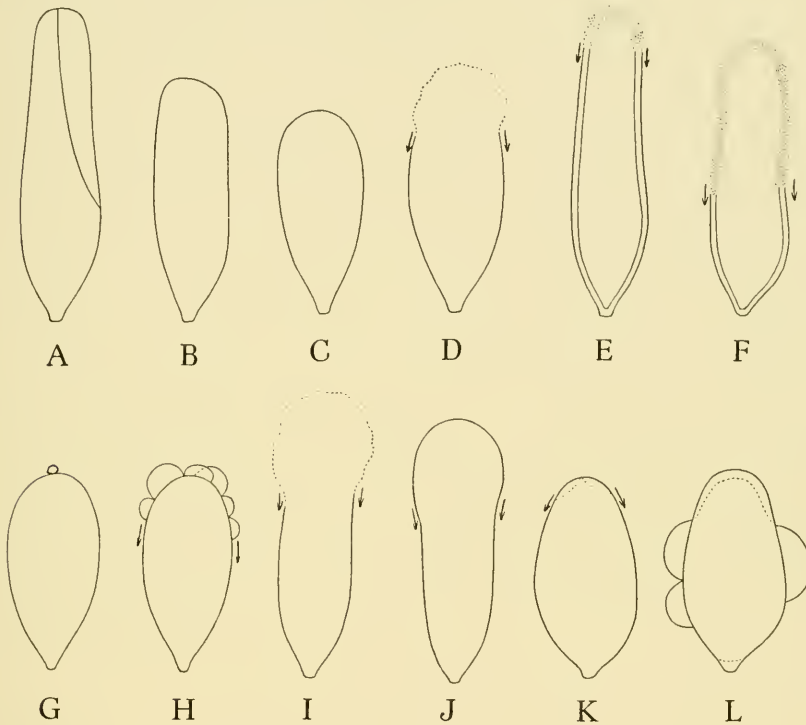


FIG. 28, *A-L*.—Differential susceptibility of *Paramecium*. *A-D*, ultra-violet; *E, F*, HCl; *G, H*, methylene blue, high concentration; *I, J*, neutral KCN; *K, L*, lack of oxygen (from Child and Deviney, 1926).

ectoplasm, progressing from the anterior end posteriorly (Fig. 28, *D*), or with less intense effect by a similar gradient of loss of structure in the ectoplasm and gradual approach to spherical form without destruction of the pellicle. In a certain range of concentration of HCl a wave of complete cytolysis of ectoplasm runs from anterior to posterior end (Fig. 28, *E, F*). In methylene blue and other basic dyes in toxic concentrations the anterior region of the ectoplasm gradually becomes more deeply stained than other

³ Child, 1914a; Bills, 1924; Child and Deviney, 1926.

parts, then shortens, and clear vesicles appear on its surface, first anteriorly, later elsewhere (Fig. 28, *G, H*). In neutral cyanide anterior shortening is followed by complete ectoplasmic cytolysis, progressing from the anterior end posteriorly (Fig. 28, *I*), or in somewhat lower concentrations by ectoplasmic disintegration within the pellicle (Fig. 28, *J*). In death from lack of oxygen anterior shortening is followed by appearance of clear areas within, or clear vesicles on the surface, these changes also progressing from the anterior end posteriorly (Fig. 28, *K, L*). In view of the findings that cyanide does not greatly decrease oxygen uptake in *Paramecium*, ectoplasmic differential susceptibility to this agent and to lack of oxygen is of particular interest (see Appendix III, p. 736). The fact that the same susceptibility gradient appears with both strong and weak acids and bases, ultra-violet radiation, and lack of oxygen is also of interest, as indicating that differential permeability is not the chief factor in determining the gradient. An ectoplasmic gradient of osmophilic substance, decreasing from the posterior region anteriorly, was found by Parke (1929) in *P. caudatum*. The anterior vacuole of this species has a more rapid rhythm than the posterior (Child and Deviney, 1926; Unger, 1926); but in *P. aurelia* the posterior vacuole is more rapid, and in *P. calkinsi* the rate of both is the same. These species differences in relative rate of vacuoles are perhaps associated with difference in the gradient of the different species and differences in form of body. Susceptibility and reduction gradients appear to be more strongly marked in *P. caudatum* than in *P. aurelia*, but in general the posterior vacuole apparently receives fluid from a larger part of the body than the anterior; this may be a factor in determining its higher rate of contraction in some species.

Other ciliates—*Frontonia*, *Spirostomum*, *Dileptus*, and *Woodruffia*⁴—show an anteroposterior ectoplasmic gradient of dye reduction. The gradient of *Dileptus* is particularly interesting; the whiplike, highly motile ectoplasmic organ at the anterior end reduces methylene blue much more rapidly than the rest of the body, if not too deeply stained, and is also much more susceptible than other parts to toxic action of dyes (Child, 1934*b*). *Spirostomum* is highly susceptible to dyes; but when slightly stained with methylene blue, a slight reduction gradient appears; with deeper staining this is reversed in direction in consequence of differential injury by the dye. The susceptibility gradient in these forms parallels the reduction gradient, except that in some individuals of *Spirostomum* a second posteroanterior gradient appears in the posterior region. *Spirostomum*

⁴ Child, 1934*b*, except unpublished data on *Woodruffia*.

moves backward almost as frequently as forward; consequently, the possibility suggests itself that this secondary gradient, evident only in some individuals of a lot, may, like the reversed oxidation gradient of *Paramecium* (p. 91), be associated with frequent backward locomotion. Cytolysis of *Stentor coerules* in cyanide progresses over the body in a wave from the peristome, and in *Vorticella* and *Carchesium* the differential in the body is similar. The contractile stalks of these animals, however, show evidence of specific susceptibility to certain agents. According to Merton (1929), their susceptibility to formol is less than that of the peristomial cilia, but greater to lactic acid, pilocarpin, and alcohol; indications of specific susceptibility of the contractile fiber of the stalk in these forms have also been observed with other agents (Child). This fiber, capable of extremely rapid contraction, undoubtedly is a highly differentiated part of the cell; it is not surprising, therefore, to find it specifically susceptible to particular agents. All hypotrichous ciliates examined for differential susceptibility, with one exception,⁵ showed anteroposterior gradient. Anteroposterior susceptibility and indophenol gradients were observed in a *Monocystis* from a marine polyclad.

The susceptibility gradient of holotrichous, heterotrichous, and hypotrichous ciliates is evident, not only in change of form and cytolysis but also in retardation and cessation of ciliary movement, which progresses from the anterior end posteriorly, except in those individuals which show a posteroanterior gradient in the posterior region; in these this gradient appeared in the cilia.

The evidence for the existence of a gradient pattern in axiate protozoa has been presented in considerable detail because presence in the morphologically differentiated part of a single cell of a spatial physiological pattern showing essentially the same characteristics as are found along the axes of multicellular animals, at least during earlier developmental stages and often throughout life, is significant as indicating that organismic pattern is independent of cell boundaries. The same physiological features of pattern appear within the single cell and in the individual consisting of millions of cells. That an axiate, or, more strictly speaking, polar, protozoan pattern consists only of these gradients in the fully developed animal is certainly far from true, but that they are essential factors in the develop-

⁵ *Stylonychia*, *Oxytricha*, *Onychodromus*, *Kerona*, and *Euplotes* all show an anteroposterior ectoplasmic gradient. In a Japanese species somewhat similar to *Uronychia* a posteroanterior gradient was observed. Whether this is associated with the very large and highly developed posterior cirri, as seems probable, or with some other factor could not be determined, since only a few individuals were found and since attempts at cultivation were not successful.

ment of the morphological pattern appears beyond question. They are evidently concerned in reconstitution of individuals in fission or after experimental isolation of parts. Moreover, it may at least be questioned whether axial co-ordination of ciliary beat and definitely directed locomotion are possible without a gradient pattern.

SPONGES, COELENTERATES, AND CTENOPHORES

SPONGES

Oxygen uptake and CO_2 production are, in general, greater in apical than in basal pieces of the sponge commonly known as *Grantia*.⁶ According to Hyman and Bellamy (1922), the oscular region is galvanometrically negative to more basal levels. In longitudinally split individuals of *Grantia* and *Leucosolenia* and several other elongated species with single osculum, the rate of reduction of permanganate and the resulting depth of color decrease from the osculum basipetally.

HYDROZOA

Gradients of early embryonic stages and their changes in the course of development, as indicated by differential susceptibility and reduction of permanganate and in some cases by the indophenol reaction, are known for several species (Child, 1925a). The oöcyte of the calyptoblast hydrozoan *Phialidium gregarium* is attached in the gonad by one pole, the future vegetal pole, the free pole becoming the animal or apical pole (Fig. 29, A). Polar bodies form at the apical pole, the first cleavage furrow progresses from it, and it becomes the apical pole of the early blastula and planula and the high end of the primary gradient (Fig. 29, B, C, D). In the later planula a second gradient appears at the original basal end (Fig. 29, D); the primary gradient becomes less distinct; the planula, if in good condition, attaches itself by the original apical end; and the secondary basal gradient becomes the hydranth-stem gradient (Fig. 29, E). In the later development of the branching hydroid the growth form is sympodial, that is, each new hydranth bud is only temporarily the apical member and becomes a lateral branch when the next bud appears (Child, 1919d). The reversal of polarity and gradient in development of the hydranth from the planula may be regarded as the first step in sympodial development; the first hydranth bud arises as a new axis from the basal regions of the plan-

⁶ Oxygen uptake higher in eleven of thirteen determinations on different lots; CO_2 production higher in ten of eleven determinations (Hyman, 1925).

ula. The same gradient relations have been found during development of two other calyptoblast species. The planula of a Japanese species, *Sertularella minurensis*, develops the secondary gradient in the original basal region at a somewhat earlier stage; in the fully developed planula the end originally basal is already more susceptible than the apical end, and the secondary gradient has extended over most of the body length.

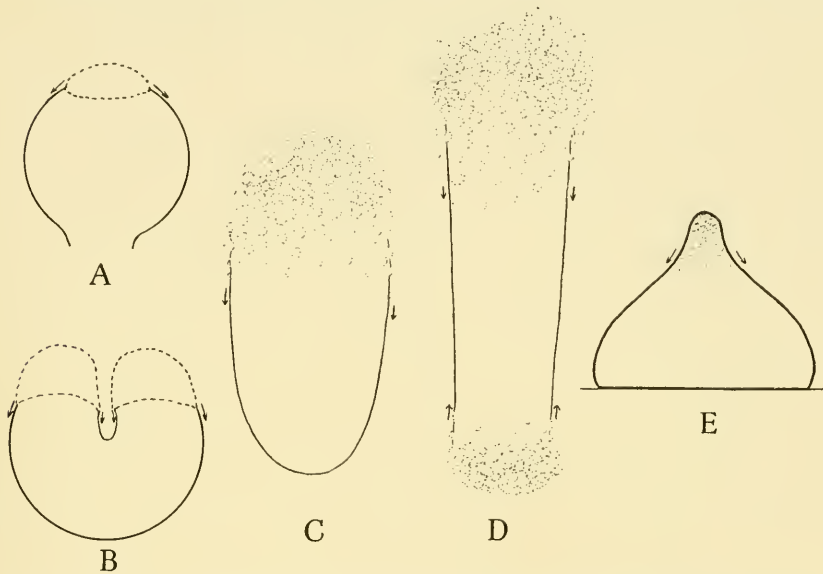


FIG. 29, A-E.—Susceptibility gradients in development of the hydrozoan *Phialidium*. A, oöcyte isolated by teasing; B, first cleavage (in A and B disintegrated portion indicated in broken line); C, early planula with gradient from apical end; D, later planula with second gradient at original basal end; E, after attachment by original apical end; secondary gradient of basal end becomes hydranth-stem axis. Arrows show direction of progress of disintegration (from Child, 1925a, with modifications).

The egg of the gymnoblast hydroid *Corymorpha palma*, when shed, sinks and adheres firmly to any solid substratum. A gradient is present in early stages with high end at the free pole, but it has not been possible to determine whether the egg orients itself in sinking or whether the gradient results from the differential in conditions from the free pole to the pole in contact. The planula never swims but may progress a short distance in contact with the substrate with apical end in advance and secreting perisarc as it goes. In the early planula differential susceptibility, permanganate reduction, methylene blue reduction, and the indophenol reaction

agree in indicating a single gradient with high end apical (Fig. 30, *A*). Somewhat later a secondary gradient appears at the basal end but remains short (Fig. 30, *B*) and becomes the region of holdfast origin (Fig. 30, *C*), while the hydranth develops at the primary apical end.

Determinations of oxygen uptake, comparative estimations of CO_2 production, differential reduction of methylene blue and permanganate, and electric-potential differences all give evidence of gradient pattern in adult hydrozoa. *Corymorpha palma* is exceptionally favorable material for re-

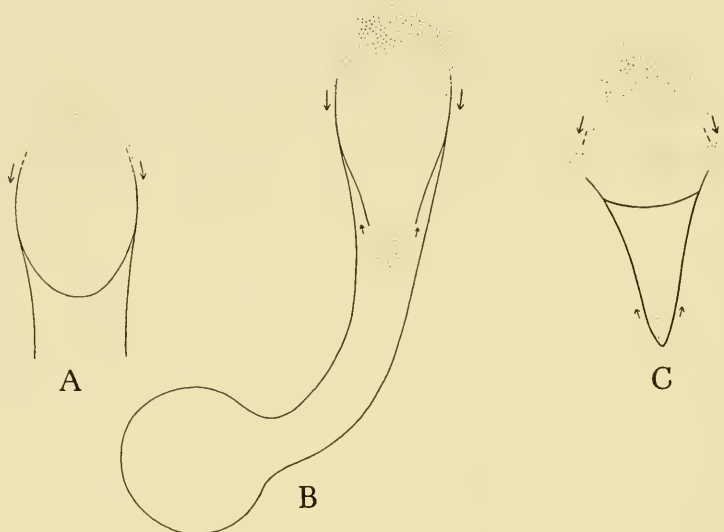


FIG. 30, *A-C*.—Development of the hydrozoan *Corymorpha palma* from early planula. *A*, early planula with single basipetal gradient; *B*, later planula with secondary acropetal gradient in basal region; *C*, young hydroid, primary gradient becomes hydranth-stem axis (from Child, 1925*a*).

spiratory determinations because of large size—it may attain a length of 10–12 cm.—and because of absence of perisarc over most of the stem length and complete absence of branches.⁷ Determinations of oxygen uptake at different levels of the naked stem region are given in Table 3, and comparative data on CO_2 production in Table 4.

Other CO_2 estimations showed a much higher rate in hydranth than in stem, and in short distal than in short middle pieces. The question of significance of these data and criticisms advanced are discussed in Appendix I (p. 729). The respiratory gradient parallels the gradient of methylene

⁷ Hyman, 1923*a*; Child and Hyman, 1926.

blue reduction and the susceptibility gradient, which concern the ectoderm only. The respiration of the large supporting cells which fill the interior of the *Corymorpha* stem is probably extremely low. Determinations of oxygen uptake in stems of *Tubularia* (Hyman, 1926a) and *Obelia* (Lund,

TABLE 3
OXYGEN UPTAKE OF DISTAL AND PROXIMAL HALVES AND DISTAL
MIDDLE, AND PROXIMAL THIRDS OF THE NAKED STEM
OF *Corymorpha palma*

(From Child and Hyman, 1926)

HALVES				
DETERMINATION NUMBER	HOURS FROM SECTION TO DETERMINATION	RESPIRATION PERIOD IN HOURS	OXYGEN UPTAKE PER GRAM PER 24 HOURS IN CUBIC CENTIMETERS	
			Distal	Proximal
1.....	20	21	0.97	0.70
2.....	20	21	0.99	0.93
3.....	18	22	1.89	1.20
4.....	18	22	2.19	1.89
5.....	16	24	3.69	2.69
6.....	7	22	1.42	1.17
7.....	7	22	2.08	1.48
8.....	7	22	1.72	1.53

THIRDS					
DETERMINATION NUMBER	HOURS FROM SECTION TO DETERMINATION	RESPIRATION PERIOD IN HOURS	OXYGEN UPTAKE PER GRAM PER 24 HOURS IN CUBIC CENTIMETERS		
			Distal	Middle	Proximal
1.....	16	23	1.63	1.28	1.15
2.....	16	23	1.65	1.46	1.46
3.....	20	23	2.66	2.06	1.75
4.....	20	23	2.78	2.18	1.98
5.....	20	24	4.86	3.04	2.62
6.....	20	24	7.01	3.82	2.15

1928c, 1931a) also show a decrease basipetally; and, according to Lund, oxygen uptake shows a differential susceptibility to cyanide; that is, the decrease in oxygen uptake by a given concentration of KCN is greater in apical than in basal pieces. Barth (*Biol. Bull.*, 78, 1940) also finds a basipetal decrease in oxygen uptake in *Tubularia*.

TABLE 4

COMPARATIVE CO₂ PRODUCTION OF DIFFERENT STEM REGIONS OF
Corymorpha palma COLORIMETRICALLY ESTIMATED WITH
 PHENOLSULPHONEPHTHALEIN AS INDICATOR
 (From Child and Hyman, 1926)

Estimation Number	Body Length in Centimeters	Region	No. of Pieces	Weight in Grams	Hours from Section to Estimation	Hours pH Change from 8.1 to 7.3
1	10-12	dist. 1/2	6	0.729	18	4:45
		prox. 1/2	6	0.7285		5:20
2	10	dist. 1/2	5	0.2525	43	7:30
		prox. 1/2	5	0.2845		9:10
3	8-10	dist. 1/2	7	0.2398	48	8:30
		prox. 1/2	7	0.2522		9:30
4	6-7.5	dist. 1/2	12	0.3005	17:30	4:00
		prox. 1/2	9	0.3057		6:05
5	5	dist. 1/2	9	0.1557	18	6:15
		prox. 1/2	8	0.1614		8:00
		dist. 1/3	18	0.2042		6:00
6	6-7.5	mid. 1/3	11	0.2073	20	8:15
		prox. 1/3	12	0.2212		8:35
		dist. 1/3	20	0.2333		5:15
7	6	mid. 1/3	17	0.2397	18	7:15
		prox. 1/3	15	0.2512		8:30
		dist. 1/3	22	0.1696		6:30
8	6-7	mid. 1/3	19	0.1743	18	7:50
		prox. 1/3	14	0.1717		11:00
9	6-7.5	mid. 1/3	11	0.1369	22	9:00
		prox. 1/3	6	0.1425		10:45

In regard to data on differential dye reduction in adult hydrozoa, the use of methylene blue with *Pelmatohydra oligactis*, the common brown hydra, shows reduction progressing basipetally in each tentacle and basipetally from the hypostome to, or almost to, the more slender stalk. In unattached animals the foot usually reduces earlier than the stalk, and reduction progresses acropetally over more or less of the stalk. In attached animals or advanced buds attached to the parent the stalk usually reduces later than the body proper. Motor activity, local contractions of body or stalk, may alter the reduction gradients to a considerable degree. In unattached animals the stalk is usually more or less motile, contracting and extending frequently, and reduces more rapidly than when inactive. When an animal bends strongly to one side while reduction is proceeding, the color may disappear completely from the contracted side and reappear when relaxation occurs. This local reduction accompanying or following contraction, with reoxidation of the dye on relaxation, has been observed repeatedly.

Methylene blue reduction progresses basipetally in each tentacle of *Corymorpha* and in the hydranth body if not altered by contraction. That oxygen uptake of the hydranth is relatively great is indicated by occurrence of dye reduction in open solutions of dye in the hydranth body and the proximal regions of the crowded tentacles if the hydranth is left without change of position for even a few moments. Frequent change of position or increase of oxygen about the hydranth by agitation of the medium brings about reoxidation. The dependence of hydranth reconstitution on oxygen tension in the related genus, *Tubularia*, indicates similar conditions as regards oxygen uptake of the hydranth (Barth, 1938b). Reduction also progresses basipetally in the ectoderm of the naked *Corymorpha* stem, except for a slight increase in rate of reduction in the extreme proximal region. From the basal region of the stem, inclosed in perisarc, numerous holdfast stolons arise as buds and elongate with growing region at the free end; in each of these rate of reduction decreases from the tip basipetally (Child and Watanabe, 1935b). In isolated pieces of the *Corymorpha* stem, rate of reduction adjoining a cut end is increased following section, and a gradient of decreasing rate extends from the cut end for a greater or less distance. The region from which the hydranth will develop later is the high region of this gradient, and within a few hours after section the length of the hydranth primordium is indicated by a well-defined region at one or both ends of the piece, where reduction is more rapid than

elsewhere. Even at this stage, before any morphological evidence of hydranth development appears, it can be determined, from the presence of a region of rapid reduction at one or at both ends of the piece, whether it will develop as a unipolar or a bipolar form (Fig. 31).⁸

Electric-potential gradients have been observed in a considerable number of hydroids.⁹ Mathews, Hyman, and Bellamy and Child and Hyman found apical levels galvanometrically electronegative to more basal levels.

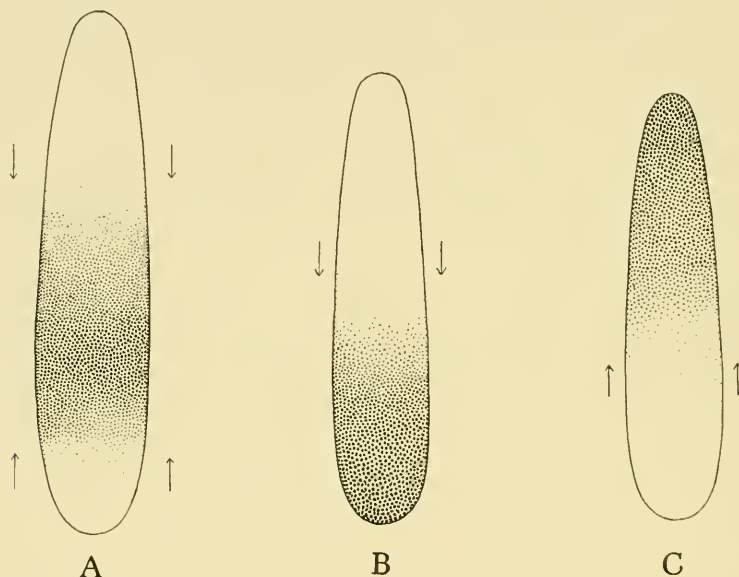


FIG. 31, A-C.—Differential reduction of methylene blue in isolated pieces of *Corymorpha* stem. A, bipolar form; B, unipolar with distal hydranth; C, unipolar with proximal hydranth. Arrows indicate direction of progress of reduction (from Watanabe, 1935c).

According to Lund, however, galvanometric positivity decreases from the apical region basipetally and increases again toward the basal end of the main axis of *Obelia*. Lund's data concern cut pieces without hydranths. Barth does not find uniformity of potential difference in different species or in the same species under different conditions. The hydranth of *Tubularia* is galvanometrically negative to middle levels, both distal and prox-

⁸ Child and Watanabe, 1935b; Watanabe, 1935c.

⁹ Mathews, 1903, *Parypha* (*Tubularia*), *Pennaria*, *Campanularia*; Hyman, 1920b; Hyman and Bellamy, 1922, *Tubularia crocea*, *Obelia geniculata*, *O. borealis*, *Schizotricha tenella*, *Eudendrium ramosum*, *Pennaria tiarella*; Lund, 1922, 1923a, 1924b, 1925, 1926; Rosene and Lund, 1930, *O. commisuralis*; Child and Hyman, 1926, *Corymorpha palma*; Barth, 1934b, *Tubularia*, *Pennaria*, *Eudendrium*.

imal cut ends of a piece are negative to the middle region, a cut end is negative to the hydranth, and a distal cut end is negative to a proximal but may become positive during reconstitution. In *Pennaria* pieces an end undergoing reconstitution is usually externally negative to other regions; in *Eudendrium* it is usually positive. It does not appear from Barth's data whether the potential of a developing hydranth differs from that of a developing stolon or whether reconstitution of the *Tubularia* hydranth by reorganization of cells already present and reconstitution by regeneration, that is, by outgrowth of new tissue from the cut end before hydranth development, may account for some of these species differences. At present the data appear to indicate that, in general, the apical region is externally electronegative in intact hydroids with fully developed hydranths; in forms like *Tubularia* and *Corymorpha*, in which hydranth reconstitution occurs by redifferentiation of a part of the stem without outgrowth of new tissue, the reconstituting hydranth is usually externally negative to other regions; in forms like *Obelia*, in which outgrowth of new tissue resembling a stolon precedes hydranth development at its free end, this growing region is externally positive. Moreover, it is possible that the sign of potential difference between the outgrowth and other levels of the piece may differ according as the outgrowth is stolonical in character or a hydranth-stem axis, and the potential of outgrowing tissue may differ from that of the differentiating or differentiated hydranth. Further investigation is necessary to determine whether these suggestions have any value; but concerning the presence of electric-potential differences as characteristics of axiate pattern in hydroids there can be no doubt. Lund regards the potentials as associated with, and dependent on, oxidation-reduction. He has shown that in *Obelia* the axial potential difference can be reversibly decreased or reversed in direction by cyanide, ether, and chloroform—in other words, the axial potential exhibits a differential susceptibility to these agents.

In several species of hydromedusae Hyman and Bellamy found the distal end of the manubrium most strongly negative externally, the margin of the umbrella next, then the subumbrellar surface, and the exumbrellar surface positive to other parts.

The galvanotactic reaction of hydra and of various hydroids and medusae shows definite relation to the gradient pattern of the body or part concerned.¹⁰

The gradients indicated by differential susceptibility and differential

¹⁰ Hyman, 1932b; Bancroft, 1904; Hyman and Bellamy, 1922.

reduction of permanganate in the body of hydra, in tentacles, hydranths, stems, and stolons of hydroids, in medusa buds and fully developed medusae parallel closely those observed by other methods. They parallel the electric-potential gradients, but apical regions which have been found to be externally electropositive and those electronegative to other regions appear as regions of high susceptibility and high rate of permanganate reduction. Assuming that the data on potential differences are correct and that the direction of sign change may result from different kinds of physiological activity in different species, particularly in reconstitution, this lack of correspondence as regards direction of gradient is to be expected, since differential susceptibility and differential reduction apparently indicate merely differences in rate or intensity.¹¹

It is a point of particular interest that early stages in the form of buds of hydranths, tentacles, medusae, and stolons appear as local gradient systems, at first more or less radial from a center and becoming longitudinal as outgrowth proceeds. In the naked contractile hydra, however, the high end of the bud gradient is not always continuously apical. Both the reduction and the susceptibility gradient of the bud may be reversed by contractile activity of the parent body, which often involves the basal region of the bud. Contractile activity of the stalk in detached animals increases its susceptibility, and bending of the hydra body may increase susceptibility of the contracted side; these effects parallel those observed in dye reduction (p. 101).

In branching hydroids with more or less definite growth form of the whole (*Pennaria*, *Obelia*, *Gonothyrea*) the multiaxiate system shows a basipetal decrease in susceptibility similar to that in multiaxiate algae (p. 86). In these cases susceptibility of the terminal hydranths decreases from the apical hydranth of the system basipetally and also basipetally in each branch, but with occasional irregularities. This gradient is in the same direction as the gradient in rate of hydranth reconstitution observed in another species of *Pennaria* (pp. 38, 39).

The gradients in a monophyid siphonophore are of interest. The coenosome or stolon arises from one side in the median plane of the bilaterally symmetrical nectomedusoid. Groups of zooids, each consisting of a nutritive zooid or manubrium with tentacles developing near its base, a medusoid, and a bract, develop successively from the region of origin of the

¹¹ For data on differential susceptibility and permanganate reduction see Child and Hyman, 1919; J. W. MacArthur, 1921; Weimer, 1928, hydra, several species; Child, 1926a, *Corymorpha*; Child, 1919a, d, 1921d; Hyman, 1920b, various hydroids and medusae.

coenosome; and successive separation of the groups occurs at the tip, the medusoid of the group becoming the nectomedusoid of a new system and giving rise to a new coenosome. The developing zooids on the coenosome show the usual radial gradient system of buds which becomes longitudinal with outgrowth. Susceptibility and reduction gradients in the subumbrellar ectoderm of the fully developed nectomedusoid are bilateral, that is, susceptibility and rate of reduction decrease from the side opposite the coenosome to the region where it develops. This bilaterality appears as a gradual change from the basipetal gradient of the earlier medusoid bud and is evident before morphological bilaterality is distinguishable in the developing medusoid attached to the coenosome. That side of the bud toward the attached end of the coenosome becomes the high end of the bilateral gradient; and the highest region on that side is next to the velum, that is, the polar gradient is not entirely obliterated. Susceptibility and rate of reduction increase with development of motility in the zooids. The definite and constant relation of the bilaterality of the nectomedusoid to the axis of the coenosome or stolon to which it is attached suggests that it originates in a gradient along the coenosome axis. The medusoid originates as a bud at right angles to the coenosome axis; and the side of the bud toward the attached, physiologically younger end of the coenosome becomes the high side of the bilateral gradient. The polar gradient of the medusoid originates from the radial gradient system of the early bud, which becomes longitudinal with outgrowth.

OTHER COELENTERATES

Susceptibility and rate of reduction decrease basipetally in the ectoderm of the *Aurelia* scyphistoma and the sessile scyphozoan, *Haliclystus* (Child). A gradient in the indophenol blue reaction has also been shown to be characteristic of polarities originating in reconstitution of the stalk of *Haliclystus* (Watanabe, 1937). Oxygen uptake of exumbrella and mesogloea of the scyphomedusa *Cassiopea xamachana* is only about 25 per cent of that of the intact animal at rest; since respiration of the mesogloea is extremely low, the exumbrellar epithelium evidently has a much lower respiration than that of the subumbrella. How much of the difference is due to absence of entoderm in the exumbrella is not known (McClendon, 1917). Developmental stages of an alcyonarian "sea pen" from early cleavage to the first polyp show basipetal decrease in rate of dye reduction.

Few data concerning actinians are available. Young individuals of

Epiactis and *Peachia* show basipetal decrease in susceptibility and rate of reduction in each tentacle and in the body. In colorimetric determinations of CO₂ production of distal and proximal pieces of the actinians *Sagartia luciae* and *Metridium marginatum* no definite difference was found (Parker, 1929), but the data have little or no significance.¹²

Entodermal susceptibility and reduction gradients in these coelenterates appear, in general, to be the same as the ectodermal. In well-fed hydras and hydroids the entoderm of the apical region may cytolize before the ectoderm; but in other regions entodermal susceptibility is apparently about the same as that of the ectoderm, except locally in the region of recently ingested food undergoing digestion. In starved animals entodermal susceptibility may be distinctly less than that of the ectoderm. Entodermal dye reduction usually follows ectodermal reduction and progresses in the same direction. In the solid tentacles of hydroids and in the *Corymorpha* stem with its core of supporting cells of entodermal origin it can usually be seen that dye reduction is most rapid in the outer parts of the entoderm cells, those parts next to the ectoderm. Respiration is probably very low in these cells, and dye reduction in them may be largely an induced result of the much higher oxygen uptake of the ectoderm. The question whether there is an intrinsic gradient in the entoderm of the hydrozoa remains open.

CTENOPHORES

Differential susceptibility of *Mnemiopsis*, *Bolinopsis*, *Pleurobrachia*, and *Beroe* decreases, in general, from the apical region in the oral direction with increase in the oral region of *Pleurobrachia* and in the lobes of the lobate forms, *Mnemiopsis* and *Bolinopsis*. The swimming-plate rows also exhibit a very marked gradient in the same direction, but this may be altered by differences in motor activity of the plates at different levels of a row. In the higher, more rapidly lethal concentrations of various agents in which movement of all plates is completely stopped within 1–2 hours, cessation of movement and death of the plates and their bases with change from the transparency of the living plates to opaque white begin at the aboral (apical) end of the row and progress orally from plate to plate. In

¹² The possibility of stimulation of the pieces following section or of laceration of the foot in detaching the animals is not considered; time between section and determination is not given; and only one set of determinations on each of four lots is presented. Moreover, since the foot is a secretory organ, it may have a high CO₂ production, and this may be increased by detachment, even without laceration. Determinations on at least three pieces—apical, middle, and basal—must be made and repeated at different periods after section before any conclusions can be drawn. See Child and Watanabe, 1933.

somewhat lower, but still finally lethal concentrations, the impulses from the apical nervous organ decrease in frequency so long before the more oral regions of the row are appreciably injured by the agent that these latter regions become physiologically isolated (pp. 327-28) and develop a vigorous beat entirely independent of the apical region, while the aboral part of the row is much less active or its plates have ceased to beat. If these conditions persist for an hour or more before death, the more active plates toward the oral end of the row may die before the less active or inactive aboral plates. The change of the plates to opaque white at death is apparently due to coagulation of their colloids. When movement is inhibited soon after exposure to the agent and death occurs in an hour or two, the base of each plate becomes white earlier than the plate itself, and the change in appearance progresses from base to tip of the cilia composing the plate. When active movement of plates continues for several hours during exposure to the agent, with final death, coagulation begins at the tips of the cilia and progresses basipetally in each plate, the plate base being the last to undergo the change.

The lobate genus *Bolinopsis* is extremely sensitive and often undergoes complete and practically instantaneous disintegration when subjected to sudden shock. Disintegration may be induced by sudden exposure to certain chemical agents, but with different concentrations or rates of addition of the agent to sea water the degree of disintegration may be varied and controlled. In concentrations near the lower limit of shock effect disintegration may occur only after several seconds or even 1 or 2 minutes. In such cases disintegration begins at the apical (aboral) pole and at about the same time at the tips of the oral lobes and progresses orally over the body and aborally in the lobes. It may progress from the apical pole over a fourth or a third of the body and stop, leaving the rest of the body, or all except the tips of the lobes, intact. In short, the disintegration gradient following shock is the same as the death gradient in slowly lethal agents which do not produce shock effect (Child, 1917c, 1933a). The ctenophore plate row is a particularly good example of a gradient with functional expression showing relations of dominance, subordination, and physiological isolation and under some conditions even reversal in direction, essentially similar to relations and alterations in developmental gradients. The reversal in direction of the death gradient in the cilia of the plate in relation to quiescence and motor activity is another feature of interest. The changes in the ctenophore plate row do not result in altered morphological development, but they do bring about altered functional development.

As regards gradient pattern and its changes, it will perhaps appear more clearly in following chapters that there is considerable similarity between this functional pattern and physiological patterns of development.

Transplantation and reconstitution of pieces of *Mnemioopsis* give evidence of a persistent polarity but not of a gradient in time of reconstitution in pieces from different body-levels.¹³

TURBELLARIA

PLANARIANS

Because of their capacity for reconstitucional development, planarians have been extensively used by many investigators as material for various lines of experiment. The relation of reconstitucional pattern to body-level of origin of the piece, as described in chapter ii, indicates that axiate pattern of the parent body plays an essential part in determining the pattern of reconstitution in the isolated piece. How far this is true will appear more clearly in following chapters. Assuming that it is true, it is obvious that information concerning the physiological characteristics of planarian axiate pattern may be expected to aid in analysis of reconstitution and perhaps of other forms of development.

Determinations of oxygen uptake in pieces from different body-levels of *Dugesia dorotocephala* by the Winkler method, with due care to eliminate, as far as possible, sources of error and complicating factors, give definite and consistent results.¹⁴ Determinations on pieces of different lengths at different periods after section showed that, following section, rate of respiration of planarian bodies cut into short pieces (1/8) is much higher than when cut into longer pieces (1/3) and that the longer pieces show only a slightly higher rate than bodies with only head and posterior end removed. Averages from the determinations in Hyman's Table 1 are: for bodies with head and posterior end removed, 1.40 cc. of oxygen per

¹³ Coonfield, 1936a, b, 1937a, b; Coonfield and Goldin, 1937.

¹⁴ Hyman, 1923b. Since an earlier study of the effect of feeding and starvation on the respiration of this species (Hyman, 1919b) had shown that a marked increase in oxygen uptake occurs on feeding, and that, in absence of food, oxygen uptake decreases in about a week to a level which remains almost constant for some weeks following, and since size of the digestive tract differs at different levels, the animals for determination were kept without food 6-18 days in order to eliminate, as far as possible, effects on total respiration of differences in activity in different parts of the digestive tract. Animals of the same length, from one stock and without sex organs, and pieces as nearly as possible the same length were used in all lots to be compared. In all determinations head and posterior end were removed so that a cut surface was present at each end of each piece. Each determination was repeated on from seven to eleven different lots of material. See Appendix I (p. 720).

gram per hour; for thirds, 1.51, and for eighths, 2.06. It may be noted here that differences in susceptibility to cyanide show similar differences in long and short pieces following section. It was also determined that in 1/8 pieces from anterior and posterior regions of the anterior zooid, that is, just posterior to the head and in the mouth region, the rate of oxygen uptake is about the same a short time after section, while 23–26 hours after section rate of the posterior pieces is distinctly lower than that of the anterior pieces. Here, again, susceptibility to cyanide shows the same likeness immediately after section and lower susceptibility of posterior pieces

TABLE 5
OXYGEN UPTAKE OF ANTERIOR AND POSTERIOR HALVES OF
THE ANTERIOR ZOOID AND A PIECE OF THE SAME LENGTH
FROM THE POSTERIOR ZOOID REGION OF *Dugesia doroto-*
cephala; TEMPERATURE $20^{\circ} \pm 1^{\circ} \text{C}$.

(From Hyman, 1923b)

NUMBER OF EXPERIMENT	DAYS SINCE FEEDING	CUBIC CENTIMETERS OF OXYGEN CONSUMED PER GRAM PER 24 HOURS		
		Anterior Half of First Zooid	Posterior Half of First Zooid	Piece from Posterior Zooid
1.	14	4.77	4.29	4.86
2.	15	6.65	5.92	6.92
3.	16	6.68	6.00	6.72
4.	14	4.88	4.28	4.52
5.	15	6.99	5.91	6.73
6.	13	5.77	5.49	5.55
7.	18	5.76	5.29	5.60
8.	12	5.18	4.32	5.01
9.	14	5.06	4.75	5.44
10.	15	6.55	5.66	6.50

later. These experiments show, first, that respiratory rate is increased in short pieces and little or not at all in long pieces following section; second, that in pieces from the posterior region of the anterior zooid oxygen uptake immediately following section is about equal to, but after 24 hours lower than, that in anterior pieces of the same length.

Table 5 gives data on oxygen uptake of pieces of equal length from three regions of the planarian body, anterior and posterior halves of the anterior zooid, and pieces of equal length from the posterior zooid region. Pieces of this length show no marked change in respiration during the first 24 hours following section; consequently, the experiments were

allowed to run 15–24 hours, and the oxygen uptake was calculated per gram per 24 hours. In all ten lots anterior halves of the anterior zooid consumed more oxygen than posterior halves; and posterior zooid pieces, about the same as the anterior pieces. Susceptibility to cyanide gives similar results.¹⁵

Colorimetric estimations of CO_2 production on pieces of *D. dorocephala* gave results essentially similar to those of Hyman as regards increase of respiration following section in shorter pieces and presence of a gradient in the anterior zooid (Robbins and Child, 1920). From colorimetric CO_2 determinations on pieces of *D. tigrina* Parker (1929) concluded that this species shows no evidence of a gradient; but, as a matter of fact, his data agree with and confirm those of Hyman and Robbins and Child.¹⁶

Because of their pigmentation and susceptibility to basic dyes, most planarian species are not favorable material for observation of dye reduction; but reduction can be followed readily on the unpigmented, or only slightly pigmented, ventral surface of a number of pigmented species. In *D. dorocephala* dye reduction (Janus green) occurs most rapidly, and at about the same time, anteriorly and in the posterior zooid region and progresses posteriorly in the anterior zooid, the last region to reduce being postoral, just anterior to the fission zone. In animals long enough to have a second posterior zooid the posterior part of the posterior zooid region may show slightly more rapid reduction than its anterior part. Differen-

¹⁵ Dr. R. M. Fraps permits mention of unpublished data obtained with a respirometer devised by him (Fraps, 1930) possessing certain advantages for this type of experiment. These data confirm Hyman's determinations as regards difference of respiratory rate at different body-levels in *Dugesia*.

¹⁶ Parker finds no definite difference in anterior and posterior halves. None is to be expected, because this species, like *D. dorocephala*, has a posterior zooid region with a higher respiration than the posterior part of the anterior zooid. Table 5 shows that a piece from the anterior region has about the same oxygen uptake as one of equal length from the posterior zooid. In another series of experiments, using the second to the fifth 1/6 pieces, anterior and posterior sixths being discarded, the average rates of CO_2 production in milligrams per gram per minute are: second 1/6, 0.00721; third, 0.00694; fourth, 0.00702; fifth, 0.00711. Second and third pieces are in the anterior zooid, and CO_2 production is less in the third than in the second; the fourth sixth includes a part of the second zooid, and its CO_2 production is consequently higher than that of the third. The fifth piece is wholly in the posterior zooid region, perhaps even in a third zooid (see pp. 41, 321) and has a still higher CO_2 production. These results are exactly according to expectation on the basis of the other data on respiration and data on head frequency and susceptibility to cyanide. The only question is whether the differences of Parker's data are large enough to be significant. Whether the animals used in these experiments were sexually mature is not known. The presence of the genital cloacal complex posterior to the mouth may increase the respiration of this region over that in asexual animals. Determinations of oxygen uptake of planarians by Shearer are discussed in Appendix I (p. 730).

tial reduction in the unpigmented *Procotyla*, a form without posterior zooids, progresses from head to posterior end, except that the extreme posterior tip may reduce somewhat more rapidly than levels slightly anterior to it. The curves of Figure 32 indicate diagrammatically the differences in the reduction gradients of *Dugesia* and *Procotyla*. They are intended merely to show roughly the differences in rate of dye reduction, as indicated by decoloration of methylene blue or change to red of Janus green

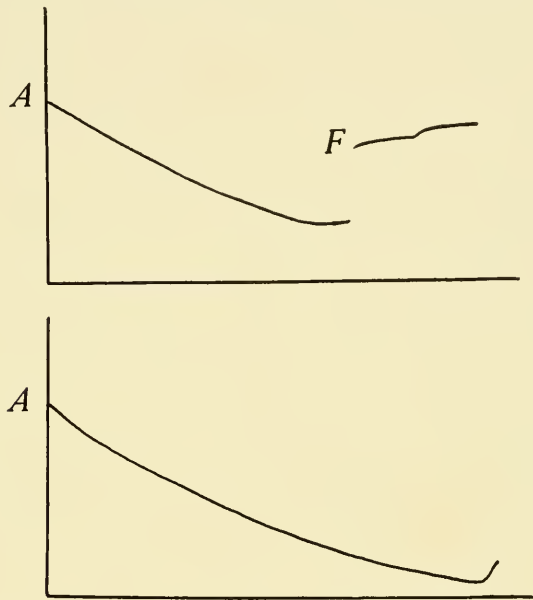


FIG. 32.—Diagrammatic curves without quantitative significance, indicating differential dye reduction in *Dugesia dorocephala* (upper) and *Procotyla fluviatilis* (lower). A, anterior end; F, level of fission; ordinates indicate degrees of decoloration or color change; abscissae, body-levels.

at a stage when reduction is about completed in the head region. In the upper curve for *D. dorocephala* the rate decreases from the head to the fission zone, where it increases rather abruptly and remains high in the posterior zooid region. The curve for *Procotyla* (below) indicates a continuous decrease in rate from anterior end posteriorly, except at the posterior tip.¹⁷

¹⁷ With slow staining, planarian ectoderm is rather susceptible to basic dyes; cytolysis usually begins in the head region, while the body is only slightly or not visibly stained. With relatively high concentrations (e.g., 1/20,000 Janus green, 1/5,000 or higher methylene blue) and with rapid oxygen decrease during staining, it is possible to demonstrate the reduction gradients on the ventral surface before cytolysis begins (Child, unpublished).

Differential susceptibility of planarians to many agents has been determined (see Appendix III, p. 734). In species without posterior zooids and fission susceptibility of ectoderm and body wall, as indicated by disintegration or other death changes, progresses from the head region posteriorly over the whole body length, except that the extreme posterior tip is usually somewhat more susceptible than regions immediately anterior to it, agreeing in this respect with results of dye reduction. Species developing posterior zooids and undergoing fission show a less simple longitudinal susceptibility gradient.¹⁸ In these forms one or more posterior zooids are more or less clearly distinguishable by their susceptibility, though not morphologically. Two, three, or even more posterior zooids are often present in long individuals of *D. dorotocephala*, and under certain conditions fission of any one can be induced (Child, 1910a, 1911d).

In gradually lethal concentrations or intensities above the limit of tolerance death of the anterior zooid progresses from the head posteriorly, but lateral margins are more susceptible than the median region to alkaline and irritating agents (Fig. 33, A-D) and less susceptible to agents which kill without stimulation or irritation (Fig. 33, E-G). The differences between margin and median region suggest a certain degree of specific susceptibility of the margins, perhaps associated with the presence there of numerous gland cells; these are apparently stimulated to secretion by alkaline and irritating agents. Dorsal and ventral epithelium and pharynx also show indications of specific susceptibility. To some agents the dorsal, to others the ventral, epithelium is more susceptible, and the pharynx is highly susceptible to certain agents but no more susceptible than other parts at the pharyngeal level to others. In general, the posterior zooid region is distinctly more susceptible than the posterior part of the anterior zooid (Fig. 33, A-D); but under certain conditions—for example, in neutral or slightly acid cyanide—its relative susceptibility is decreased (Fig. 33, E-G), and it may be less susceptible than the anterior zooid. In these respects the differences between anterior and posterior zooids are very similar to those between old and young individuals.

Susceptibilities of isolated pieces to cyanide differ with length of piece and time after section. Pieces one-third to one-fourth the total body length of animals 15 mm. or more in length show little or no increase in susceptibility after section, except immediately adjoining cut surfaces. Simi-

¹⁸ Species used for observation of differential susceptibility are as follows: without posterior zooids and fission: *Procotyla fluviatilis*, *Fonticola velata*, *Phagocata gracilis*, *Curtisia foremani* (see p. 41, footnote 7); with posterior zooids and undergoing fission at a definite zone: *D. dorotocephala*, *D. agilis*, *D. tigrina*, and a Japanese species, externally very similar to *Dugesia*.

lar fractions of body length from small, young animals usually show increase. With decrease in relative length of piece, increase in susceptibility after section becomes greater. Moreover, in pieces of equal length from the anterior zooid the increase is greater with increasing distance of the piece from the anterior end; susceptibility of pieces from the posterior part of the anterior zooid becomes as high as, or even higher than, that of anterior pieces. Pieces from the posterior zooid region resemble anterior pieces in showing less increase in susceptibility than posterior pieces of

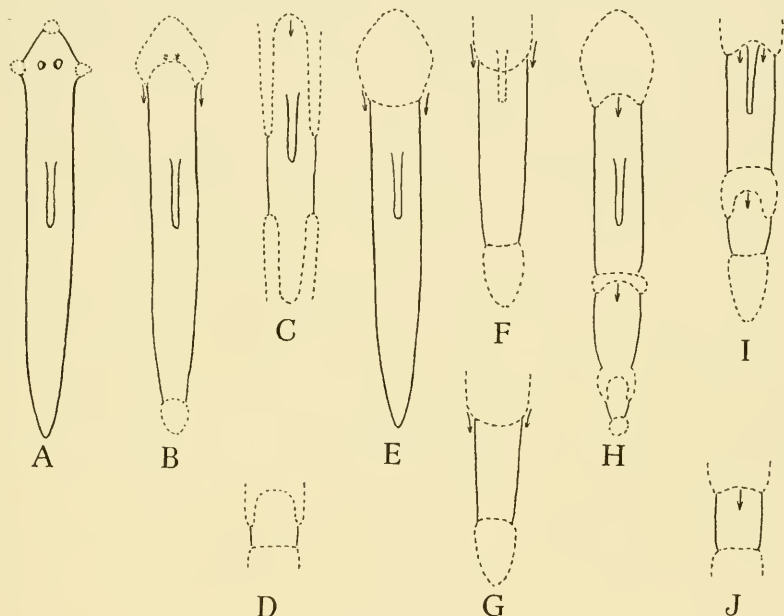


FIG. 33, A-J.—Differential susceptibility of *Dugesia dorotocephala* in KCN. A-D, m/1,000, alkaline; E-G, m/1,000, neutral or slightly acid; H-J, m/10,000, alkaline. Arrows indicate direction of progress of disintegration.

the anterior zooid. In general, the effect of section on these shorter pieces is obliteration or, in shorter pieces, inversion of the susceptibility gradient of intact animals. The posterior region of the anterior zooid becomes equally susceptible to, or more susceptible than, other regions. These alterations of susceptibility are temporary and apparently represent a stimulation of the pieces following section. They gradually disappear, the gradient in pieces becoming similar to that of intact animals after 12 hours or more (Child, 1914c). That they indicate real, though temporary, changes in physiological condition at different body-levels, in relation to

the general gradient pattern, seems evident. They are also of interest because degree of inhibition of head regeneration in pieces parallels the increases in susceptibility following section; and if the increases are prevented, head development is not inhibited (see pp. 177, 406). The changes in rate of oxygen uptake found by Hyman are, so far as determined, essentially parallel to the changes in susceptibility.

In low concentrations which kill very slowly but are still above the limit of tolerance (e.g., KCN m/10,000), more than one posterior zooid, if present, may become distinguishable by slight differences in susceptibility (Fig. 33, *H*, *I*, *J*). As regards the death gradient of the digestive tract, it is difficult to attain certainty, for direct exposure, at least to chemical agents, depends more or less on disintegration of the body wall. There is, however, some evidence that in well-fed animals death progresses from the pharyngeal region anteriorly and posteriorly. In agents which penetrate readily, such as cyanide, the gut of well-fed animals disintegrates as early as, or earlier than, the body wall; but in starved animals it is much less susceptible and may still be intact after the body wall has disintegrated.

In the earlier stages of reconstitution the new tissue at both ends is more susceptible than the old, and the developing head more susceptible than the posterior tissue; in the old tissue susceptibility decreases from the head posteriorly. When a head develops at the posterior end of a piece or in any other than the usual position, a longer or shorter susceptibility gradient arises in relation to it.

Attention must be called to the fact that the anteroposterior gradient pattern in postembryonic stages of the planarian body does not represent a gradient of growth in body length. The rate of growth increases posteriorly, as is at once evident from comparison of individuals of different length; Abeloos (1928) has given statistical data concerning this point. The physiological gradients are not necessarily growth gradients and may or may not parallel such gradients (see Needham, 1931, p. 584).

Evidence of differential tolerance and differential acclimation or conditioning has been obtained with *D. dorotocephala*. In low concentrations of ethyl alcohol (1-1.5 per cent) most individuals remain alive and intact for at least a week or two and show more or less evidence of increased tolerance or acclimation. During the first few days they are more or less anesthetized, but motor activity increases after several days of exposure. Small young individuals, whether from eggs or reconstituted pieces, while more susceptible than large individuals to higher concentrations of alcohol and at first less active than large in these low concentra-

tions, acclimate more rapidly and more completely than the large animals. They become active earlier and show less differential death than the older individuals. After a week or two disintegration usually begins to appear in the posterior region of the anterior zooid in some of the larger animals, that is, just anterior to the level of fission, and progresses anteriorly from this region (Fig. 34, *A*). The body gradually becomes separated into two independent pieces, the posterior zooid region and the anterior part of the anterior zooid (Fig. 34, *B*). After progressing a greater or less distance anteriorly in the anterior zooid, the disintegration may cease, and healing of the wound and regeneration of a new posterior end may occur slowly in the same concentration that brought about the disintegration (Fig. 34, *C*). The posterior zooid may also remain active and slowly regenerate a new head (Fig. 34, *C*). In a number of individuals of the same length in the same container, differences in the time of disintegration and the rate and amount of its progress anteriorly occur. Some individuals may disintegrate completely (the head region and the posterior zooid last of all), while in others disintegration is limited to the posterior part of the anterior zooid, and both anterior piece and posterior zooid or zooids regenerate and remain alive indefinitely. Small, physiologically young animals usually remain alive and intact

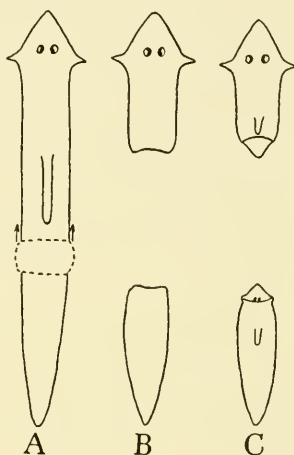


FIG. 34, A-C.—*Dugesia dorotocephala*, differential tolerance and differential acclimation.

in concentrations in which partial death of the large, old individuals occurs; in slightly higher concentrations they may show a similar partial death. The range of concentrations giving these results differs with temperature, nutritive condition, and size of animals and must be determined experimentally for a particular stock. Susceptibility to toxic and lethal effects of these low concentrations increases with decrease and decreases with increase of temperature, the reverse of susceptibility to concentrations above the limit of tolerance.

Occasionally heads only, or heads and a short anterior portion of a few of the larger animals, may disintegrate early, and a new, more or less inhibited head develops slowly. In somewhat higher concentrations this may sometimes occur in smaller animals. These cases represent the beginning of the death gradient characteristic of higher concentrations. The

physiologically old heads have a lower range of tolerance than those of young animals and are sometimes killed by the primary toxic action of the agent before they are able to acquire tolerance. If the heads do not die during the first few days of exposure to the agent, they usually remain alive indefinitely, or until starvation is far advanced, when the whole animal may die.¹⁹

In KCN $m/100,000$ most individuals live indefinitely; in KCN $m/4/100,000$ most or all die. Between these limits, however, some degree of differential tolerance appears in many individuals, with reversal of the death gradient in the anterior zooid and acclimation of more or less of the anterior region and of the posterior zooid region. Anterior ends of pieces show greater tolerance or acclimation in regeneration than posterior ends (Child, 1933*c*). In animals kept at 3°–5° C. a similar differential tolerance appears in many individuals, with separation of the body into an anterior piece and the posterior zooid by disintegration of the posterior part of the anterior zooid, and later acclimation with regeneration of a posterior end in the anterior piece and a head in the posterior piece at the same temperature.

The point of chief interest in these experiments is the reversal in direction of the death gradient and the greater tolerance to low concentrations of those regions which are most susceptible to high concentrations of the same agents. They also constitute further evidence concerning the character of the longitudinal physiological pattern of the planarian and its relation to reconstitution in pieces from different levels. The evidence at hand from oxygen uptake, CO₂ production, differential dye reduction, and differential susceptibility to lethal effects of agents suggests that the more tolerant regions, also more capable of acclimation, are more active regions, regions of higher metabolism. In the case of alcohol, tolerance apparently depends on ability to oxidize it. In animals living in low concentrations of alcohol for several weeks without feeding, the rate of oxygen uptake increases greatly, sometimes 500 per cent, though the animals are sluggish and do not lose weight more rapidly than controls in water (Buchanan, 1922, pp. 28, 29). In differential tolerance and acclimation the regions of greater tolerance and acclimation are apparently regions of higher rate of oxidative metabolism. As will appear in later chapters, evidence of differential tolerance and differential acclimation or conditioning within the in-

¹⁹ Child, 1911*e*, 1913*a*, 1914*b*. It is perhaps unnecessary to note that in these experiments on tolerance to alcohol the animals were kept in closed containers of large volume with small air space, and solutions were renewed daily or every 2 days.

dividual is to be obtained not only from postembryonic stages of planarians but from early embryonic stages of various animals and in the same relation to gradient pattern.

OTHER TURBELLARIA

Observations on the zooid chains of the rhabdocoel *Stenostomum*, in which morphological development of zooids precedes separation (Fig. 10), show that rate of methylene blue reduction and susceptibility decrease from the head region of each zooid after an early stage of zooid development.²⁰

Susceptibility, as indicated by cytolysis, and the indophenol blue reaction in eggs removed from the uteri, cleavage stages, and early larvae of

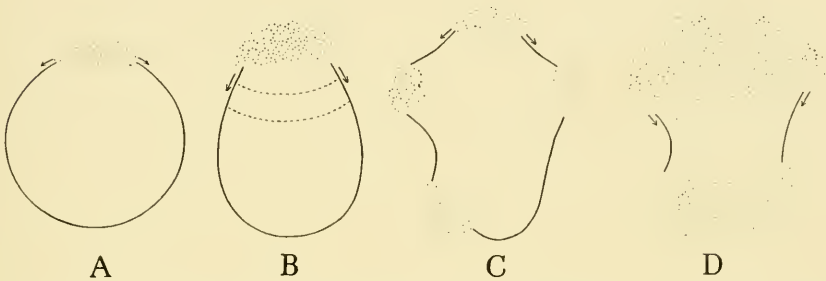


FIG. 35, A-D.—Susceptibility gradients in eggs and developmental stages of *Stylochus ijimai*. A, egg removed from uterus; B, early larva; C, D, later larval stages. The indophenol blue gradient is similar (after Watanabe and Child, 1933).

the Japanese polyclad *Stylochus ijimai* show a gradient decreasing from apical to basal pole (Fig. 35, A, B). In later larvae the apicobasal gradient persists in the general ectoderm, but the cells of the two ciliated bands also show a higher susceptibility and rate of indophenol reaction than adjoining regions (Fig. 35, C), and in the most advanced larvae examined the whole posterior region shows increased susceptibility and rate of indophenol reaction (Fig. 35, D). Conditions during metamorphosis are not known. Determinations of CO₂ production of pieces from different body-levels of adults show a U-shaped gradient in most individuals (Fig. 36). Whether the high CO₂ production of the posterior region is associated with the presence in it of the terminal genital complex is not certain. As noted above, the posterior region shows increase in susceptibility and rate of indophenol reaction in later larval stages, before there is any trace of the

²⁰ Child, 1924b, susceptibility; 1934a, dye reduction.

genital organs. Ectodermal susceptibility of adults decreases from the posterior end anteriorly, with a slight increase at the anterior end in most individuals (Watanabe and Child, 1933). The CO_2 gradient represents, of

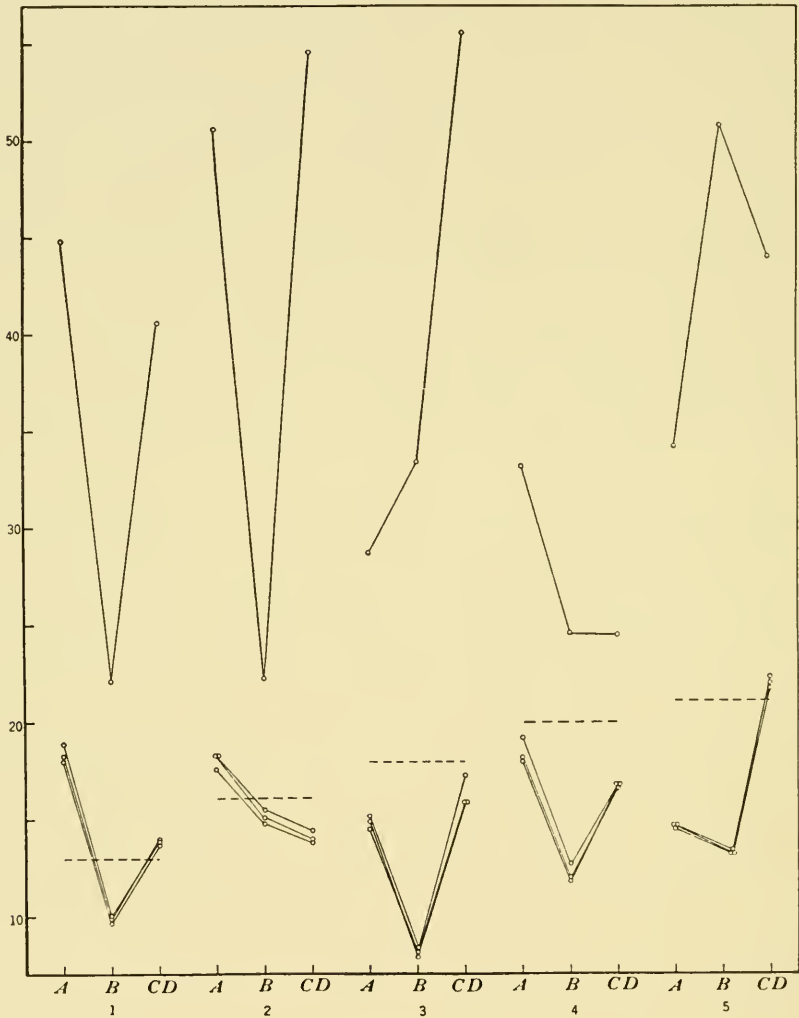


FIG. 36.— CO_2 production of *Stylochus ijimai* divided into three pieces, A, B, CD; extreme anterior and posterior ends discarded; five series of determinations, each lot consisting of three pieces from the same body-level; ordinate, thousandths of a milligram of CO_2 per gram per minute; abscissae, body-levels. Horizontal broken lines indicate CO_2 production of intact animals, upper curves, CO_2 production immediately after section, lower curves, CO_2 production 2, 4, and 6 hr. after section (from Watanabe and Child, 1933).

course, the total CO_2 production of all organs at the different levels; and the data on dye reduction in a related species, presented below, indicate that gradients in different organs differ in direction. The ectodermal susceptibility gradient gives no information concerning internal organs. However, the very slight variations in successive CO_2 determinations on the same lots 2, 4, and 6 hours after section indicate that they are physiologically significant as indicating a definite respiratory pattern of the whole.

The gradient of the earlier larval stages of a Californian *Leptoplana* is apicobasal, like that of corresponding stages of *Stylochus*. Observations on differential reduction of methylene blue in adults of this species and of a planocerid closely resembling *Stylochus* appear to throw some further light on the question of gradient pattern in these forms. When only the body wall is stained, rate of dye reduction decreases from the posterior end anteriorly and from median to lateral regions. When animals are stained throughout, internal reduction is most rapid in the cephalic ganglia and decreases in all directions from them, more rapidly posteriorly than anteriorly. In both species susceptibility of the ectoderm and body wall, like dye reduction, shows a gradient decreasing from the posterior end anteriorly. Apparently, then, there are in these forms at least two gradients—an ectodermal and an internal—in opposite directions. It is a point of some interest that the cephalic ganglia reduce more rapidly than other internal organs. When the genital complex is present, it reduces more rapidly than adjoining regions, but not as rapidly as the ganglia. The uncertainty as regards interpretation of respiratory gradients of adult animals with different, more or less localized organ systems is well illustrated by these polyclads. The different lines of evidence suggest that the primary gradient persists internally, apparently in relation to the nervous system, while secondary gradient changes occur in ectoderm and body wall. In cleavage and gastrula stages of the planocerid species rate of reduction of methylene blue in low oxygen decreases basipetally from the apical region.

ANNELIDS

The bipolar migration of alkaline and acid substances in the egg of *Nereis* at the time of polar-body formation and their accumulation, respectively, in apical and basal regions indicate the existence of an axial differential of some sort (Spek, 1930, 1934b). Spek suggests that the migration is due to an electrical factor, but the existence of such a factor implies a physiological differential.

A few data on differential susceptibility of developmental stages are available (Child, 1917*d*; Hyman, 1916*a*). Susceptibility of the egg of *Chaetopterus pergamentaceus* before and after fertilization decreases from apical to basal pole (Fig. 37, *A*). During maturation susceptibility of the apical region is relatively high. The basipetal gradient persists during cleavage and after gastrulation in the ectoderm, but in early motile stages susceptibility increases in the dorsiposterior region, slightly anterior to the extreme posterior end, and in the early trochophore this region becomes more susceptible than the apical region and extends ventrally around the larva (Fig. 37, *B*, *C*). This is the region of the so-called "somatic plate," which gives rise to the trunk segments. The region of the

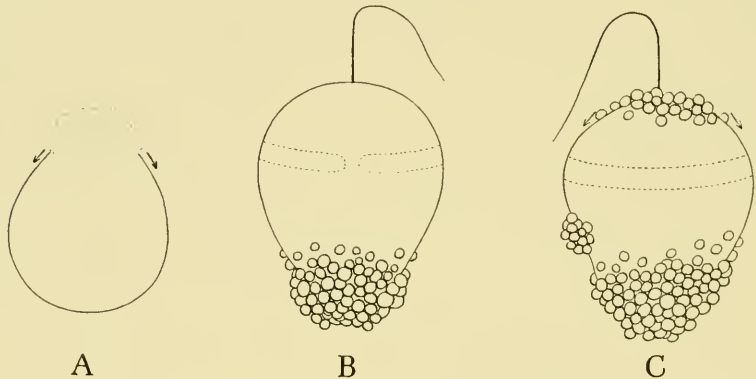


FIG. 37, *A-C*.—Differential susceptibility of *Chaetopterus pergamentaceus* egg and larvae. *A*, basipetal gradient in undivided egg; *B*, *C*, stages of cytolysis in trochophore (from Child, 1917*d*).

developing mouth also shows increased susceptibility at this stage (Fig. 37, *C*). *Nereis limbata* and *Arenicola cristata*, also polychetes, show a similar simple gradient from apical pole in early stages; later, as the somatic plate becomes active, its susceptibility increases. The cells of the *Nereis* prototroch also show increased susceptibility as their ciliary activity develops. What may be called a U-shaped gradient is apparently characteristic of annelids from, or somewhat before, the stage when development of trunk segments begins, throughout life, or as long as segment formation continues. In these polychetes it originates in the trochophore. The term "U-shaped" is merely a convenient designation for a gradient pattern in the longitudinal axis with two high ends and a low region between them; strictly speaking, such a gradient is not U-shaped but consists of two arms with differential in opposite directions. It is present at least temporarily in

other segmented animals, as well as in annelids, and also in the polyclad larva (see Fig. 35, *D*) preceding posterior elongation, though segmentation does not occur. It is apparently associated with posterior elongation of the body, whether by formation of segments or not. In early stages of embryonic elongation of *Tubifex tubifex*, an oligochete, susceptibility shows only the posterior arm, that is, it decreases from the posterior segment-forming region anteriorly without evidence of higher susceptibility in the head region (Fig. 38, *A, B*), according to Hyman (1916a). The activity of the rapidly developing segments appears to dominate the whole embryo. Somewhat later, however, when segments between the head and the posterior growing region have undergone further development, the U-shaped gradient characteristic of later, and probably also of earlier, stages appears (Fig. 38, *C*).

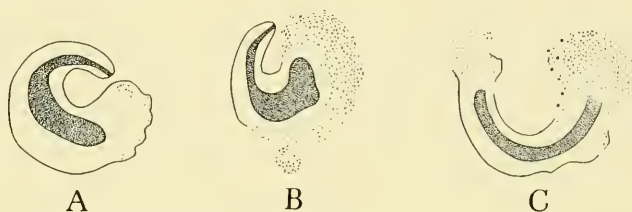
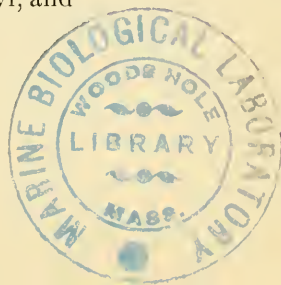


FIG. 38, *A-C*.—Differential susceptibility of embryonic stages of *Tubifex tubifex*. *A, B*, earlier, and *C*, later, stages (from Hyman, 1916a).

Respiration in relation to body-level has been determined in several annelid species, both polychetes and oligochetes. Considering the data with some departures from order of appearance, Hyman and Galigher (1921), using the Winkler method, found a U-shaped gradient of oxygen uptake, with the posterior end usually higher than the anterior, in *Nereis virens*, *N. vexillosa*, and *Lumbriculus inconstans*. Shearer (1924), using the Haldane respirometer, found the oxygen uptake of anterior pieces of an earthworm (species not given) at least twice as high as that of posterior pieces and a similar difference in the oxygen uptake of acetone powders of similar pieces. Since he used only anterior and posterior pieces, his data do not show whether a U-shaped gradient was present in the species used. In a later paper (Shearer, 1930) he concludes that his earlier data were incorrect but presents no new determinations on the earthworm. Perkins (1929) found a slight U-shaped gradient of oxygen uptake in earthworms (*Lumbricus* sp.; *Allolobophora* sp.) but noted that it did not correspond with the gradients of total iodine equivalence, extractable sulphhydryl, and



total sulphur. He suggested, however, that the oxygen-uptake gradient doubtless includes other oxidation systems than those associated with growth. Okada (1929) and Kawaguti (1934) found a U-shaped gradient of oxygen uptake and CO_2 production in the microdrilous oligochete *Branchiura*. Determinations of CO_2 production, of oxidizable substance by a modification of the Manoilov method, and of the temperature of onset of heat-shortening in the earthworms *Pheretima communissima* and *Allolobophora* (Watanabe, 1931) give very similar U-shaped gradients for both species after increase of CO_2 production following section has disap-

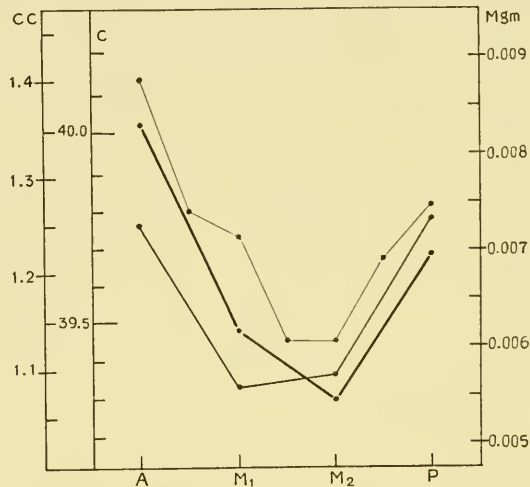


FIG. 39.—Gradient of oxidizable substance (heavy line), of CO_2 production (medium line), and of heat-shortening temperature (light line) in *Allolobophora foetida*. CC, cubic centimeters of KMnO_4 required for oxidation; C, centigrade temperature; Mgm, milligrams of CO_2 per gram per minute; abscissae, body-levels; A, anterior (from Watanabe, 1928).

peared (Fig. 39). Except for Shearer's conclusion, not based on evidence, that his experimental data were incorrect, there is essential agreement among all these investigators.

Determinations of CO_2 production on *N. virens* (Parker, 1929) give very different results. He finds rate of CO_2 production of anterior and posterior pieces lower than that of middle pieces. Examination of his data, however, shows that rates of the pieces are from 12 to 30 per cent lower than that of intact animals and that this difference is due entirely to decrease in the rates of anterior and posterior pieces after section. He finds anterior and posterior regions more susceptible than the middle region to cyanide, and his data on CO_2 production suggest that they are also more sus-

ceptible to loss of blood and experimental conditions. Since his determinations were made "a few hours" after section and were not repeated on the same lots at different periods, they are without definite significance in relation to the gradient problem, except in so far as they indicate that anterior and posterior regions have undergone depression since section.

These results with *Nereis* and the views advanced in connection with them led Hyman (1932a) to undertake a re-examination of oxygen uptake in *N. virens* with the Winkler method. She finds that during the first 3 hours after section the rates are irregular but that from 3 to 9 hours after section the U-shaped gradient is characteristic. Pieces from the posterior region undergo rapid depression in physiological condition after section; consequently, she regards her data as showing a much lower rate in these pieces than the normal. Electric-potential differences and galvanotactic reaction in *Nereis* are also dependent on freshness of the material (Hyman and Bellamy, 1922).

Using extremely short pieces in order to eliminate motor activity, Malœuf (1936) maintains that there are no significant differences in oxygen uptake at different levels of an earthworm, the differences observed by others being attributed to differences in motor activity, but presents no actual evidence in support of his opinion. This work, like that of Parker, provides another illustration of the difficulties involved in determining the significance of respiratory determinations in isolated pieces of animals. The effect on respiration of loss of blood and of the large area of cut surface in relation to volume is entirely unknown. Malœuf also finds that total solids, total organic material minus fat, and fat of the body wall and of the whole starved animal, in percentages of wet weight, increase posteriorly in the preclitellar region and decrease from the clitellum posteriorly, except for an increase in fat at the posterior end. It is of some interest to note that these gradients are essentially the inverse of susceptibility and dye-reduction gradients and of the respiratory gradients found by most investigators. That such gradients are present without any corresponding differences in respiratory rate seems rather remarkable. The anterior regions contain less reduced glutathione than other regions, in which the differences are negligible.

It is sufficiently evident from these data on annelid respiration that a single set of determinations on pieces after section does not afford a basis for any definite conclusions, except that further investigation is necessary. Repeated determinations on the same lots at different times after section and determinations on pieces representing different fractions of the body

length must be made; effects of conditions favoring or stimulating motor activity and those favoring quiescence must be evaluated as far as possible before reasonable certainty can be attained as to the presence or absence and significance of a respiratory gradient independent of motor activity and other incidental factors. At present most of the evidence indi-

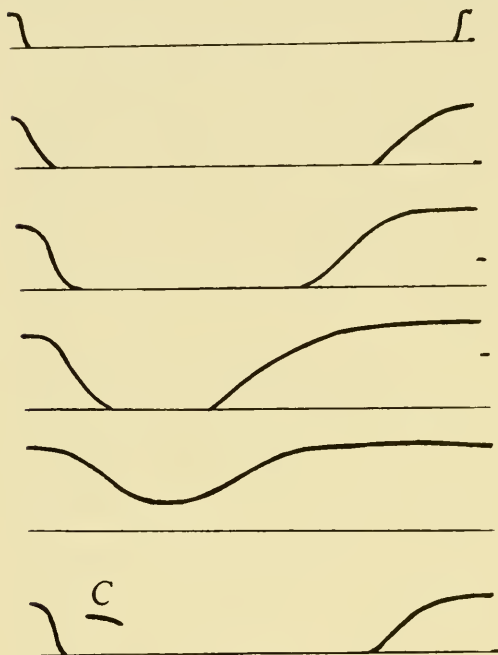


FIG. 40.—Diagrammatic, indicating successive stages of differential reduction of dyes in body wall of *Tubifex tubifex* when not appreciably injured by the dye. Ordinates indicate degree of reduction; horizontal base line, no visible reduction; anterior end at left; diagram at bottom indicating reduction in animal with well-developed clitellum, *C* (from Child and Rulon, 1936).

cates presence of a U-shaped respiratory gradient and is in agreement with results of dye reduction, susceptibility, electric-potential difference, and galvanotactic reactions, some of which are obtained in complete absence of motor activity.

Observations on differential dye reduction in several microdrilous oligochete species and several earthworms show a U-shaped reduction gradient with posterior arm much longer than anterior, except in certain forms after segment development is completed and the posterior growing region disappears. In zooid chains of *Nais* and *Acolosoma* each new zooid develops a reduction gradient with rate decreasing posteriorly from the head region and anteriorly from the posterior-segment-forming region, when that is present and

active. In *Acolosoma* the posterior arm of the gradient is present only temporarily, since the posterior growing region disappears after a certain number of segments is formed, and the gradient in the fully developed individual becomes a simple gradient, decreasing from the head region to the posterior end (Child, 1934a; Child and Rulon, 1936).

Stages of differential dye reduction in *Tubifex* are indicated diagrammatically in Figure 40. Reduction is most rapid anteriorly and posterior-

ly, except that the anal segment, indicated by the short heavy line on the base line at the right, reduces slowly (Fig. 40, top). The anal segment, of course, is not a part of the posterior growing region but is the original posterior end of the body. It is interesting to find it so sharply marked off from the growing region immediately anterior to it.

A stage of reduction in animals with well-developed clitellum (*C*) is indicated in the diagram at the bottom of Figure 40. The clitellum is sharply distinguishable from adjoining regions by its much higher rate of reduction. With overstaining, differential susceptibility to the dye results in earlier injury to anterior and posterior regions than to other parts, with U-shaped gradient. Reduction is differentially re-

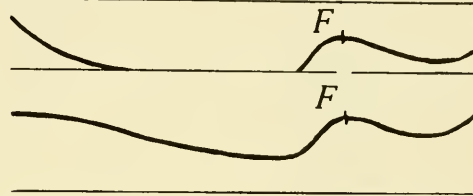


FIG. 41.—Stages of differential dye reduction in two-zooid chains of *Aeolosoma*, *Nais*, and *Stylaria*, indicated as in Fig. 41; fission zone at *F* (from Child and Rulon, 1936).

tarded in the injured regions, with the result that the normal reduction gradient undergoes complete inversion, the middle region reducing most rapidly, though no more rapidly than in normal animals, and rate of reduction decreasing both anteriorly and posteriorly.

The normal U-shaped gradient appears in actively motile animals and also in animals with motility practically completely eliminated by anesthesia, though possibly slightly less steep in the anesthetized animals. Motor activity of animals isolated for a week or two in clear water in daylight decreases greatly. These animals often show no visible movement when observed continuously during the period of dye reduction, but the reduction gradient is essentially the same as in actively motile animals. Evidently differential dye reduction does not result from differential motor activity. Differential dye reduction in two-zooid chains of *Aeolosoma*, *Nais*, and *Stylaria* is indicated in Figure 41, the fission plane being at *F*. This gradient may also be completely inverted by overstaining and differential injury of anterior and posterior regions of each zooid. Motor activity is certainly not greater in the region of fission than in adjoining regions; it is probably less, for the posterior region of the anterior zooid and the head of the second zooid are not fully developed.

In several unidentified species of earthworms a similar two-armed reduction gradient is present. In one form, however, without indication of loss of posterior segments, rate of reduction decreased from anterior to

posterior end. This was perhaps a species in which new segments are not formed after hatching (Sun and Pratt, 1931). In all forms tested for dye reduction a distinct ventrodorsal reduction gradient appears, rate of reduction decreasing from the mid-ventral region laterally and dorsally. In the more or less transparent microdrilous oligochetes the segmental ganglia are visible through the body wall in the living individual. With suffi-

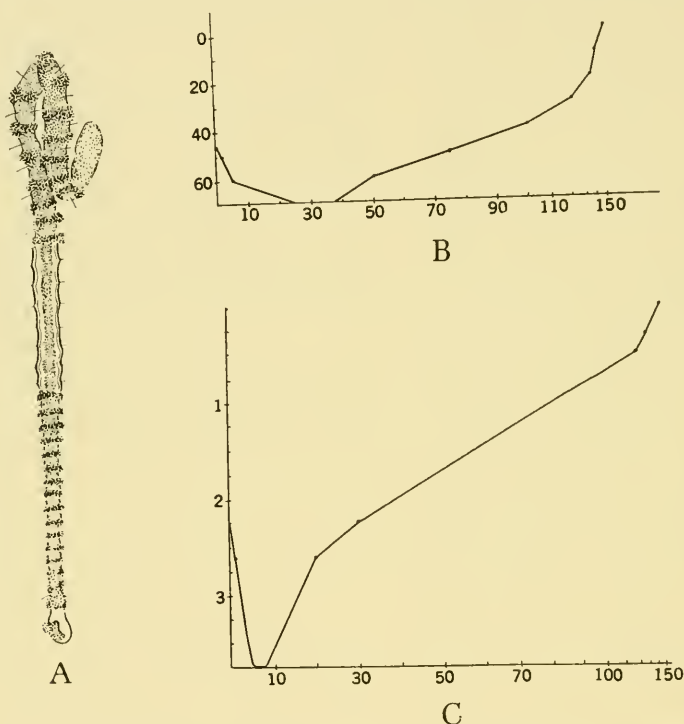


FIG. 42, A-C.—Susceptibility gradients of microdrilous oligochetes. A, *Lumbriculus inconstans*; B, *Tubifex rivulorum*; C, *Dero limosa*. In B and C ordinates indicate minutes; abscissae, body-levels as segment number from anterior end at left (from Hyman, 1916a).

cient exposure to the dye they become stained, and repeated observations seem to show a higher rate of reduction in them than in the ventral body wall. However, since they are seen through the stained body wall, the possibility of error exists.

Susceptibility gradients observed by Hyman (1916a) in microdrilous oligochetes parallel the reduction gradients. Except in the fully developed *Aeolosoma* individual, the forms examined show a U-shaped death gradient. Different species differ as regards relative length and height of anterior and posterior arms of this gradient (Fig. 42, A, B, C). The anal seg-

ment of later developmental stages is sharply marked off by a much lower susceptibility from the segment-forming region immediately anterior to it, except in individuals in which a new posterior end has recently developed in connection with fission (Fig. 42, C). In this respect, also, differential susceptibility parallels differential dye reduction. Inversion of susceptibility gradients has been observed in *Dero* and some other microdrilous species kept in clear standing water in diffuse light for a week or two. Susceptibility to cyanide is decreased but increases from the two ends toward a region anterior to the middle. Evidently the animals are differentially susceptible to the unfavorable environment, and the differential effect on physiological condition inverts differential susceptibility to cyanide.

The electric-potential gradient of earthworms tested also shows two opposed arms, at least on the ventral side, both ends being galvanometrically negative to an intermediate region (Morgan and Dimon, 1904; Watanabe, 1928). The galvanotactic reaction of the earthworm and of several species of polychetes is also significant in this connection. When exposed to the current, the animals bend into a U-shape with the two ends toward the cathode.²¹ If the current is strong or if the animals are exposed for a long time, a rather sudden reversal has been observed in a number of species, both ends turning toward the anode. If these animals are removed from the current for 15-30 minutes, they recover, and both ends are again cathodic on renewed exposure.

Water content, distribution of setae, and pigmentation show a U-shaped distribution in various species of earthworms, according to several authors; but an increase in water content from anterior to posterior levels has been reported in two species.²² The difference in temperature at which heat-shortening occurs at different body-levels has been determined for several species of annelids. The differences are characteristic for the species, showing in some a simple, in others a U-shaped, in still others an inverted U-shaped, gradient and in some a more varied differential. In the earthworms tested, these temperature gradients are U-shaped and correspond closely to the other gradient expressions. Their relations to other conditions at different body-levels in polychetes are not known; they may perhaps correspond to regional differentiations (Hatai, 1924a; Watanabe, 1928).

Few observations have been made on susceptibility gradients in adult

²¹ Moore and Kellogg, 1918; Hyman and Bellamy, 1922; Moore, 1923.

²² Hatai, 1924a; Kopenhaver, 1937, water content. Hatai, 1924b; Sivičkis, 1930, setae. Pickford, 1930, pigmentation.

polychetes. In unidentified syllids individuals developing from buds showed the U-shaped gradient after the posterior segment-forming region was established. The two ends of *N. virens* are more susceptible to cyanide than the middle.²³

If it be granted that a U-shaped gradient involving metabolism is present in the body walls of adults of a considerable number of annelid species and that the posterior arm of the gradient is related to the progressive formation and development of new segments, there is every reason to believe, as was noted years ago by Hyman (1916a), that the two arms of the gradient are not identical in kind of metabolism. The anterior arm apparently represents the final expression of the primary embryonic polar gradient in this fully differentiated region; the posterior arm represents different stages of growth and differentiation of segments, the earliest stages being anterior to the anal segment, with stages of development and physiological age progressively more advanced from segment to segment anteriorly as long as segment formation and development continue. It would be most surprising if there were not a metabolic gradient in this region during the period of segment formation and development. But in those species in which segment formation ceases, either at hatching or at some later stage, and all segments sooner or later attain full development, this posterior arm of the gradient may disappear completely, and only a simple gradient from anterior to posterior end may be present; or, with special differentiations and functions of particular regions other gradient modifications may appear. In *Tubifex*, for example, development of the clitellum alters the gradient of the body wall in the region where it appears. Determinations of oxygen uptake, CO₂ production, differential dye reduction, and differential susceptibility show only certain quantitative factors and give no information concerning other axial differences, which may be and doubtless are, present in the adult. That the metabolism indicated by oxygen consumed or CO₂ produced is the same in character in anterior and posterior regions of an annelid body does not follow from the data and is not assumed; but that there are differences in basal rate, that the rate de-

²³ Parker, 1929. This author concluded that the higher susceptibility of anterior and posterior regions resulted from entrance of cyanide through the mouth and anus. This is certainly not the case in other forms: death and disintegration begin on the outer surface of the body; the posterior arm of the susceptibility gradient of *Tubifex* and other microdrilous oligochetes extends over more than half the body length; that cyanide or other agents should be carried so far anteriorly in the intestine is extremely improbable. Moreover, differential death of the body wall may occur in vital dyes without staining of the intestine. Also, differential susceptibility to lack of oxygen is the same as to cyanide.

creases from the head posteriorly for a certain distance, and that the earlier stages of segment development in the posterior region have a higher rate than later stages at more anterior levels is indicated by a large body of evidence, even though there is not complete agreement.

EMBRYONIC AND LARVAL DEVELOPMENT OF ECHINODERMS

Embryonic development of echinoderms is one of the classic materials of developmental physiology. It has been the object of a great number of experimental studies dealing with many special problems, but all more or less directly concerned in one way or another with the problem of developmental pattern. Among the many investigations concerned with this material, the concept of a gradient or gradients of some kind as essential factors of axiate pattern has appeared in various forms. Probably the first suggestion that the polar axis is represented by a gradient was advanced by Boveri (1901). The earlier experimental studies of Runnström (1914, 1915) on echinoderm development led him to the view that the axiate pattern of sea-urchin development is based on systems of layers or concentration gradients of substances. Further development of this hypothesis by Runnström and his co-workers, particularly Hörstadius and Lindahl, will be discussed as occasion arises. A hypothesis of gradient pattern in sea-urchin development in terms of the colloid substrate has been advanced by von Ubisch (1936, 1938). Observations on differential susceptibility, as indicated both by differential death and by differential modification of development, on differential reduction of potassium permanganate and later of dyes and in the indophenol blue reaction, in other forms as well as in echinoderms, led Child to a hypothesis of axiate pattern in terms of gradients in which differences in rate of metabolism were regarded as essential factors and probably the most important in early development.²⁴

In this chapter only some of the more direct evidence on which these hypotheses are based will be dealt with.²⁵

²⁴ Child, 1913*a*, *d*; 1914*f*; 1915*a*; 1916*a*, *d*; 1928*d*; 1936*a*, *b*.

²⁵ In order to avoid possible confusion, certain axial relations in echinoderm development and the terms used in describing them are noted. The terms "animal" and "vegetal" or "vegetative," dating from the earlier days of embryology but still widely used for eggs and early embryonic stages generally, have reference to the more or less marked segregation of protoplasm and yolk and the difference in developmental activity in opposite polar regions of the eggs of many animals. The more active protoplasmic pole where polar bodies are usually formed is the animal pole. The application of these terms to echinoderm development in which polar differences are slight in early stages is largely conventional. In the following pages

OÖCYTE AND UNDIVIDED EGG

Differential dye reduction in early, growing oöcytes of echinoids²⁶ seems to indicate a very slight decrease in rate of reduction from the region where the nucleus is nearest the cell surface. This is usually, but by no means always, nearer the free than the attached pole. In full-grown oöcytes approaching maturation, with nucleus no longer visible and retaining evidence of position of stalk of attachment, a slight reduction gradient with highest rate at the pole opposite the region of attachment seems to be present: maturation stages have not been obtained. In a few unfertilized eggs after maturation a slight gradient, with most rapid reduction at the egg surface in the region opposite the pole of attachment, or in some eggs in the region where the nucleus is near the surface, has been observed; but whether the nucleus is still at the apical pole or elsewhere is not known. In all stages preceding fertilization these reduction gradients are so slight that their presence becomes reasonably certain only with repeated observations. Only uncertain indications of a possible differential susceptibility have been observed in echinoid stages preceding fertilization (Child, 1916a). A higher susceptibility to cyanide of the ventral side in the undivided egg of the sea urchin *Paracentrotus lividus* has been reported (Foerster and Orström, 1933).

The nucleus of the full-grown asteroid oöcyte usually lies near the cell surface at some point, more often nearer the free than the attached pole. With slight staining, reduction apparently decreases in rate from that part of the cortical region where the nucleus is nearest the surface; but here, as in the echinoids, the gradient is at best very slight. According to

the terms "apical" and "basal" are often used in place of them, as being more convenient. Gradients observed are often designated as "basipetal" or "acropetal," meaning that the progress of the change indicating the gradient is basipetal, toward the vegetal or basal pole, or acropetal, toward the animal or apical pole. In echinoids the apical polar region becomes the oral lobe of the pluteus larva (see Fig. 73), the basal region, mesenchyme, and entoderm; and the most basal region of the ectoderm becomes the anal side of the pluteus. The apical polar region of the asteroid starfish egg becomes the apical and functionally anterior end of the larva (see Fig. 83). The so-called "ventrodorsal axis" of the echinoid and asteroid larva is more or less nearly at right angles to the apicobasal or polar axis; that is, one side of the embryo becomes conventionally ventral, the opposite side dorsal. The ventral side of the echinoid pluteus, the side including the oral lobe and the anal arms (see Fig. 73), is functionally anterior and has sometimes been called "anterior" (Child, 1915a, 1916a, d), sometimes the "oral side" or "field"; the opposite pointed or rounded end of the pluteus, "posterior" or "aboral." In the asteroid larva, however, the ventral side is oral, and the anus comes to lie more or less ventrally.

²⁶ Material, *Strongylocentrotus purpuratus*, *Dendraster excentricus*; dyes, methylene blue and Janus green; Child, 1936a. In this paper dye concentrations and staining periods are given for all figures.

E. B. Wilson and Mathews (1895), that region of the oöcyte where the nucleus is nearest the surface becomes the apical or animal pole in *Asterias forbesii*, and the polar bodies form there. Polar bodies in this species are said by Yatsu (1910a) always to appear halfway between equator and free pole. In *Patiria miniata*, another asteroid genus, their position, like the position of the oöcyte nucleus, is highly variable, ranging from the free pole to a position between equator and attached pole (Child, 1936a, Figs. 12-14). According to Schaxel (1914; 1915, pp. 35, 36) the attached pole becomes the apical pole in *Asterias glacialis*; this is not the usual relation of egg polarity to ovarian attachment in echinoderms or other invertebrates.

When maturation of the *Patiria* egg begins, a very distinct cortical dye-reduction gradient appears, with most rapid reduction in the region of polar-body formation (Fig. 43, A). When the cell is stained throughout, reduction progresses basipetally in the interior as well as in the cortex (Fig. 43, B). The region about the vegetal pole reduces last, but little or no gradient is visible there. The susceptibility gradient with cyanide in the ovarian oöcyte of *A. forbesii* (Fig. 43, C, D) progresses from the region where the nucleus is nearest the surface through the whole cell (Child, 1915a). The oöcyte nucleus, whether in the cell or isolated, also shows a susceptibility gradient the same as that of the cytoplasm in nuclei not isolated (Fig. 43, E). Whether this actually represents a nuclear gradient or results from differential exposure of the nucleus as cytoplasmic cytolysis occurs is not certain, but the differential susceptibility of isolated nuclei suggests a real nuclear gradient. Conceivably a nuclear gradient might be determined by the differential of its position near the surface of the cell.

From experiments with hypertonic and hypotonic solutions Dalcq (1925) infers presence of a gradient of electrical charge on the plasma membrane of the egg of *A. glacialis* with basipetal increase of positivity. Under natural conditions the egg of *A. forbesii* does not show the migration of alkaline and acid substances to opposite polar regions observed in some other eggs, probably because of high viscosity of the cytoplasm, but under conditions which decrease viscosity the bipolar migration occurs (Spek, 1934c). The migration is apparently in relation to the axis of attachment of the egg in the ovary; but, if earlier investigators are correct, this is not the final polarity of the egg and embryo. Spek's description and figures indicate accumulation of acid substances at the pole of attachment, alkaline substances at the free pole.

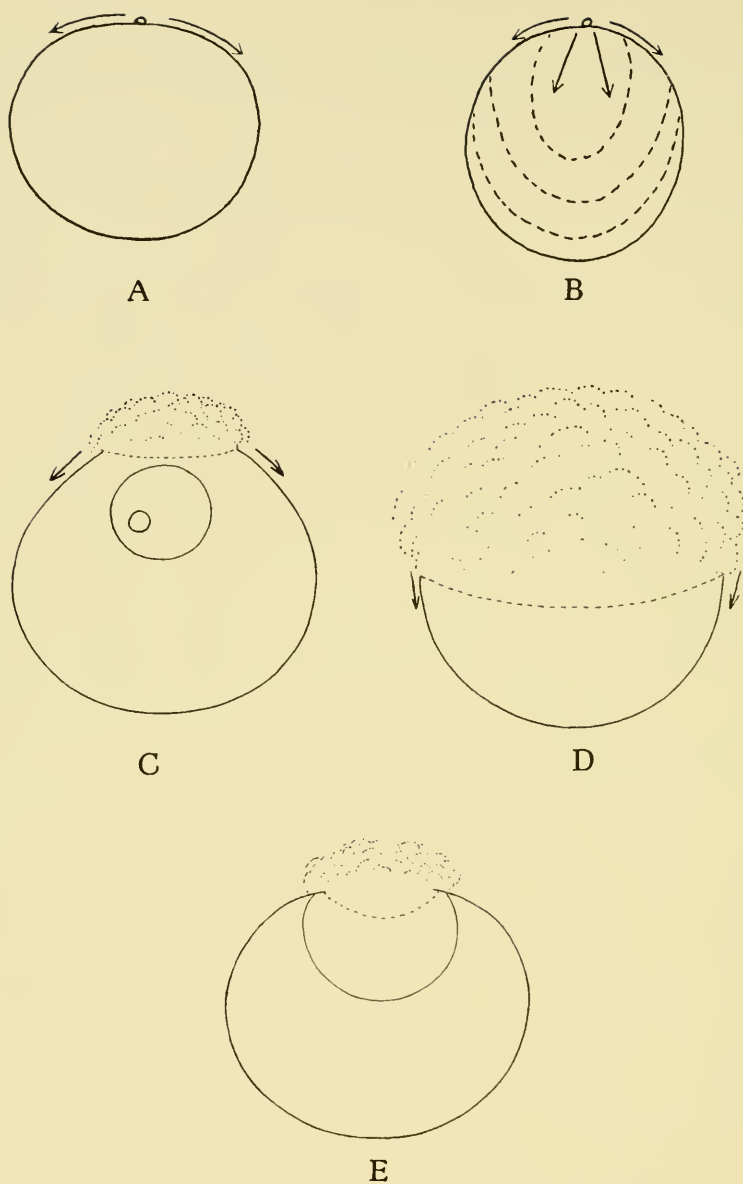


FIG. 43, A-E.—Differential dye reduction and differential susceptibility to cyanide in asteroid oöcytes; arrows indicate direction of progress of reduction and cytolysis. A, B, maturation stages, reduction progressing from apical pole (*Patiria miniata*; from Child, 1936a); C, D, differential cytolysis in ovarian oöcyte of *Asterias forbesii*; E, differential cytolysis of both nucleus and cytoplasm in *Asterias* oöcyte (from Child, 1915a).

CLEAVAGE STAGES AND EARLY BLASTULA

In early cleavage stages of *Strongylocentrotus purpuratus* and *S. franciscanus* rate of reduction decreases basipetally, but the differential is slight;

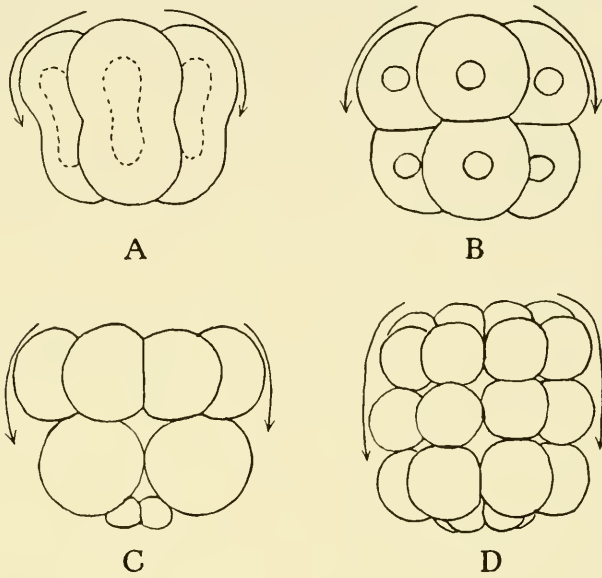


FIG. 44, A-D.—Dye-reduction gradient in early cleavage stages of *Strongylocentrotus franciscanus*; arrows indicate direction of progress of reduction (from Child, 1936a).

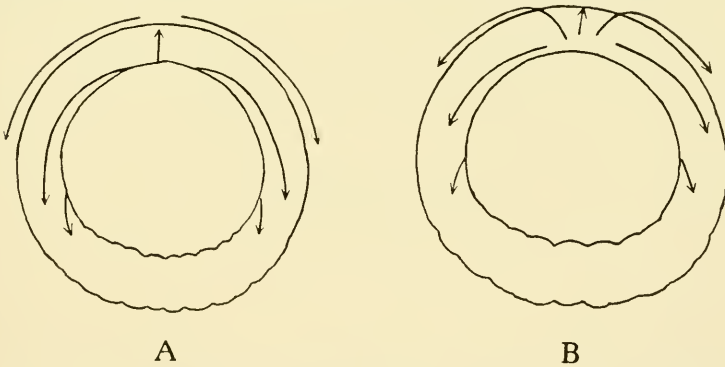


FIG. 45, A, B.—Dye-reduction gradient in early echinoid blastulae; optical sections, A, *Strongylocentrotus purpuratus*; B, *Dendraster excentricus* (from Child, 1936a).

no evidence of more rapid reduction in the micromeres at the basal pole, either during their formation or in following cleavage stages, has been obtained (Fig. 44). Results of a few observations on susceptibility of these

stages are uncertain as regards presence of a differential. In the early echinoid blastula the basipetal reduction gradient becomes more distinct (Fig. 45). At all levels reduction is most rapid in the inner ends of the cells and progresses outward; this perhaps indicates merely that oxygen tension in the blastocoel falls below that in the external medium.

Early cleavage and early blastula stages of the asteroid *Patiria* show a more strongly marked basipetal reduction gradient than the echinoids, that is, the difference in rate of reduction at the two poles is greater. The susceptibility gradient is also distinct in the early blastula and basipetal, like the reduction gradient (Child, 1915a, 1916a).

THE LATER BLASTULA

In later blastula stages a change in the reduction picture occurs. A second reduction gradient appears in the basal region, extends acropetally, and partly obliterates the primary gradient. In the echinoids the center of this secondary gradient system is the basal pole where the mesenchyme cells, products of the micromeres, lie; but the adjoining entoderm reduces almost as rapidly (Fig. 46). As immigration of the mesenchyme cells begins, they become the most rapidly reducing cells of the whole individual, and the prospective entoderm is only slightly less rapid (Fig. 46, B, E).

Formation of primary mesenchyme preceding gastrulation does not normally occur in asteroids, but a secondary gradient develops in the basal region of the later *Patiria* blastula, though usually not as strongly marked and not extending as far acropetally as in the echinoids; earlier reduction on the presumably ventral side is also evident (Fig. 47).

The earlier observations on differential susceptibility of the later blastula stages in the sea urchin *Arbacia punctulata* and the starfish *Asterias forbesii* agree with the data on dye reduction in that they show a decrease in susceptibility from the apical pole basipetally and from one side, presumably ventral, according to evidence from gastrula stages; but they do not show increase in susceptibility of mesenchyme and prospective entoderm (Child, 1915a, 1916a). More recent data on differential susceptibility to cyanide, lack of oxygen, and the dyes methylene blue and Janus green do show this increase in *Strongylocentrotus*; but the basal gradient is more narrowly limited to the mid-basal region than is the dye-reduction gradient, as comparison of Figure 48 with Figure 46 will show (see Appendix IV, p. 739). The presumably ventrodorsal differential is evident in Figure 48. In the asteroid basal increase in susceptibility does not appear until later.

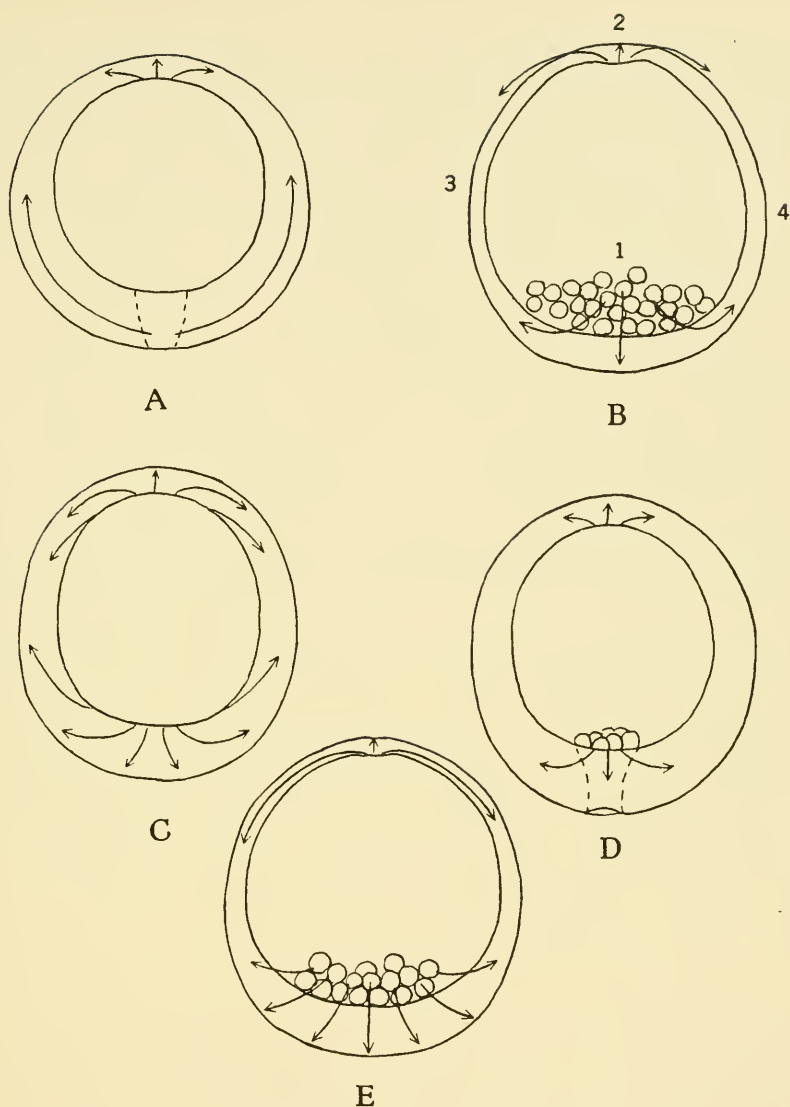


FIG. 46, A-E.—Differential dye reduction in late echinoid blastulae. A, B, *Strongylocentrotus franciscanus*, with numerals, 1-4, indicating time-order of reduction of different regions in B; C-E, *S. purpuratus*, preceding and during immigration of mesenchyme. Arrows indicate direction of progress of reduction, and an attempt is made to indicate by their lengths the relative rate of spread of reduction from the center of earliest appearance (from Child, 1936a).

The change in condition of the basal region preceding gastrulation, as indicated by dye reduction, suggests that it is undergoing activation; the changes in its developmental behavior—immigration of the mesenchyme, invagination of the entoderm—also suggest that a change of some sort has

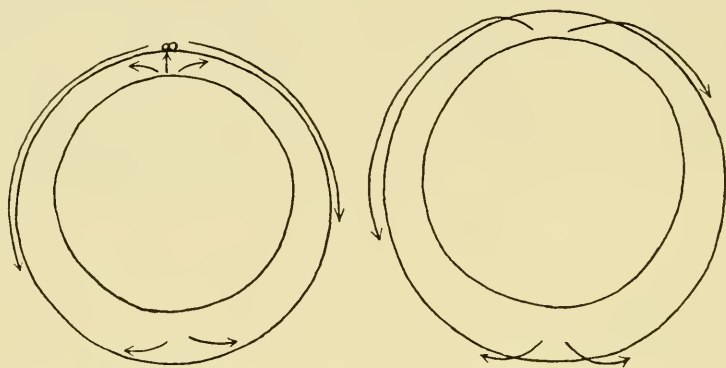


FIG. 47.—Differential dye reduction in late blastulae of *Patiria*; reduction more rapid on one side, the left in the figure, presumably the ventral side (from Child, 1936a).

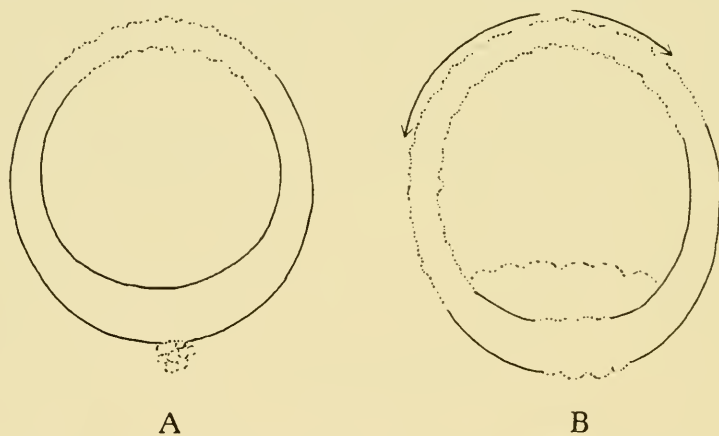


FIG. 48, *A*, *B*.—Differential susceptibility of late *Strongylocentrotus* blastulae to lack of oxygen and to cyanide. *A*, before immigration of mesenchyme only mesenchyme cytolized in basal region; *B*, after immigration mesenchyme and middle entodermal region cytolized; ventral(?) side more susceptible than dorsal (from Child, 1936a).

occurred. If the change is an activation, as dye reduction suggests, it is not necessary to assume that the two opposed gradient systems represent identical activities. Gradients involving different metabolic reactions and different protoplasmic substances may be similarly indicated by reduction or susceptibility differentials.

THE GASTRULA AND LATER STAGES

The dye-reduction gradients in early echinoid gastrulae are essentially similar to those of late blastulae. The mesenchyme in the blastocoel reduces earlier than any other cells. The entoderm follows closely; and as it elongates inward, it develops a distinct basipetal gradient, that is, the radial differential present before invagination becomes longitudinal with invagination, as in a bud. In the ectoderm rate of reduction decreases from both apical and basal poles and from the ventral side dorsally (Fig. 49, *A*). Later the beginning of development of the stomodeum and of the oral lobe from the apical region is accompanied by increased rate of reduction in those regions, and rate of reduction ventrally is distinctly higher than dorsally (Fig. 49, *B*).

As the pluteus form begins to develop, the oral lobe and the region of each anal arm show reduction, decreasing in rate from the tips. Elongation of the dorsal region is also accompanied by a slight reduction gradient, with high end at the tip. With completion of pluteus development the reduction gradients become less distinct: changes associated with metamorphosis have not been observed.

In the early gastrula of *Patiria* the invaginating entoderm does not show a high rate of reduction at its tip, but entodermal rate is highest at the blastopore, decreasing in both directions; in the ectoderm, rate decreases from apical and basal regions (Fig. 49, *C*). As enlargement and decrease in thickness of the inner free end of the entoderm begins, a new reduction gradient with high end at the tip appears, that is, the basipetal gradient appears here, but later than in the echinoid (Fig. 49, *D*). That the difference is in some way associated with formation and isolation from the epithelial layer of mesenchyme preceding gastrulation in the echinoid and at a considerably later stage (after invagination in the starfish) seems probable.

As decrease in thickness of the apical end of the entoderm progresses, with separation of mesenchyme cells into the blastocoel and development of the esophagus, the basipetal gradient becomes still more distinct (Fig. 49, *E*). The region about the blastopore, however, still shows early reduction, perhaps associated with contractile activity there, which is evident at this stage in closure and opening of the blastopore, as if a sphincter were present. The beginning stomodeal invagination is represented by a localized radial gradient system or field of rapid reduction, and the whole ventral side of the larva reduces more rapidly than the dorsal (Fig. 49, *E*). As the ciliated bands develop, they reduce somewhat earlier than the

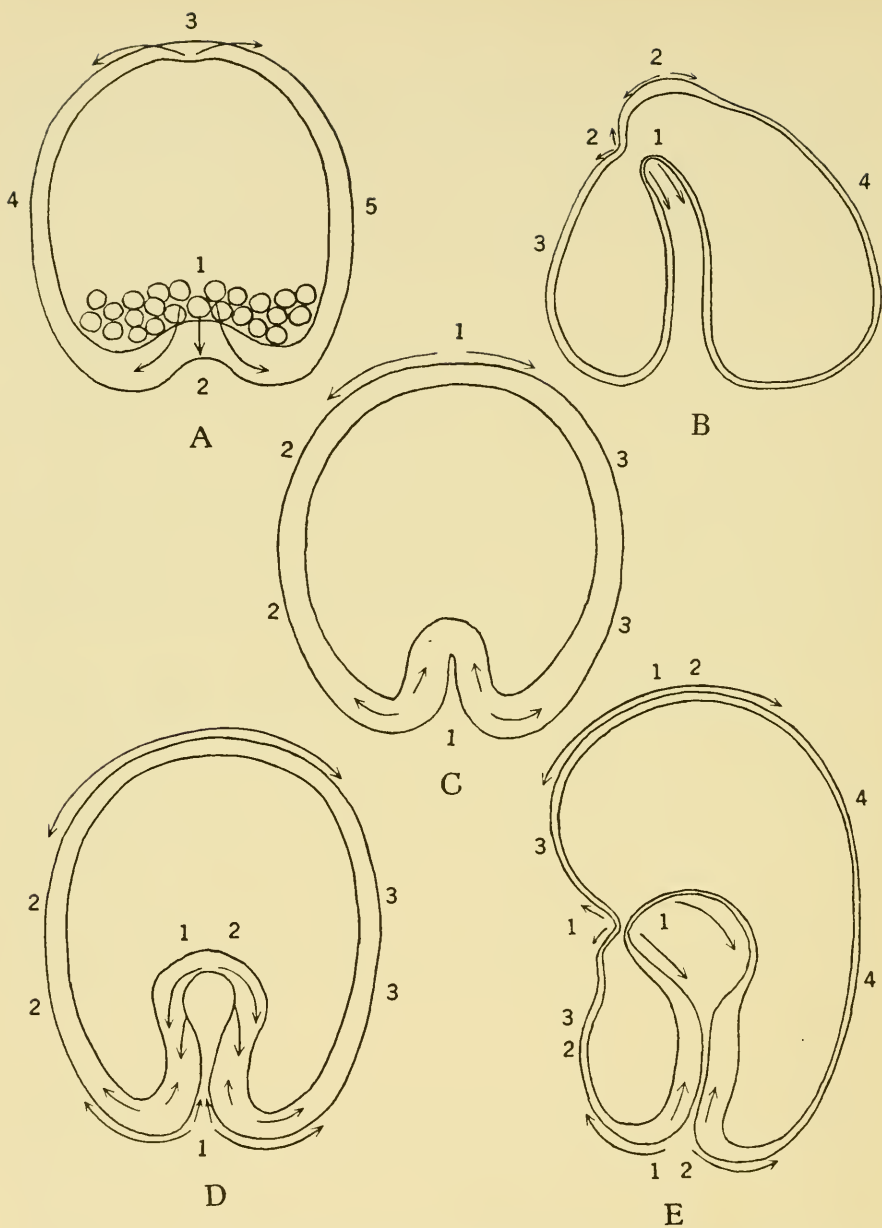


FIG. 49, A-E.—Differential dye reduction in echinoderm gastrulae. Arrows show direction of progress of reduction, their relative lengths, relative rates of progress; numerals 1-5, adjoining certain regions, indicate the general time-order of reduction, with 1 as most rapid; two numerals (1, 2; 3, 4) adjoining the same region indicate individual variations in order. A, B, *Strongylocentrotus purpuratus* (*S. franciscanus* and *Dendraster excentricus* similar); C-E, *Patiria* (A, C-E, from Child, 1936a).

general ectoderm about them. As the two coelom sacs develop from the thin-walled apical archenteron, they seem to reduce slightly more rapidly than the adjoining entoderm; but this is somewhat uncertain because the whole apical archenteron at these stages reduces very rapidly, often showing reduction while staining in open solution, probably because oxygen content in that part of the enteric cavity is below the critical level. Here, as in the echinoids, the gradients associated with development of organs become less distinct or disappear with termination of larval development. Apical and ventral regions usually still reduce somewhat more rapidly than the dorsal ectoderm. Some changes in entodermal reduction occur, probably associated with regional differentiation. Reduction during metamorphosis has not been observed.

It seems evident that these reduction gradients and their changes indicate some of the quantitative features of echinoderm developmental pattern. The changes in the gradient systems in the course of development and their close association with development of morphological form are of particular interest. However, they give no direct evidence concerning absence or presence of regional specificities or qualities, though it seems highly probable that there is a relation of some sort between them and regional differentiation. In general, the gradients appear to precede anything clearly distinguishable as differentiation.

The earlier observations on susceptibility in *Arbacia* and *Asterias* gastrulae agree with these more recent data on dye reduction, so far as ectoderm is concerned. Susceptibility was found to decrease from the apical region of the *Asterias* entoderm of middle and later gastrula stages; and, later, unpublished observations showed high susceptibility about the *Asterias* blastopore. Developing oral lobe and anal arms of *Arbacia* showed basipetal susceptibility gradients.

According to a recent study of dye reduction in development of two other species of sea urchins, dye reduction progresses from the apical pole basipetally throughout larval development, with no indication of a secondary acropetal gradient in late blastula and gastrula. It is also described as progressing from the outer cell surfaces inward, instead of from within, outward, as observed by Child (Ranzi e Falkenheim, 1937). These authors find no evidence of an indophenol blue gradient, as might have been expected, for concentration of the reagent used was so high that it certainly killed at once, and dead embryos and larvae show no indophenol gradients or only the merest traces of them. Only use of the reagents in very high dilutions will show the gradients in living animals. Also, no

gradient was observed with the nitroprusside reaction for glutathione. Although a basipetal reduction gradient appears in their material, their conclusion is that there is no actual gradient but that the basal region produces a substance inhibiting metabolism and raising redox potential; this decreases in concentration acropetally and so gives the appearance of a basipetal gradient. This substance and its effects are entirely hypothetical, no evidence of its existence being given. They, of course, do not accept the interpretations of the Runnström school or the conclusions of Spek.

OTHER DATA AND HYPOTHESES

The indophenol blue reaction with extremely dilute reagents, which permit appearance of intracellular indophenol blue in the living animals, gives a very distinct basipetal color gradient in blastulae and gastrulae of *Asterias*, supposedly indicating a gradient of active indophenol oxidase. Unfortunately, attention was not given to the question of presence or absence of a ventrodorsal gradient (Child, 1915a). Susceptibility of the sand dollar *Echinarachnius parma* to a number of agents is like that of *Arbacia* (J. W. MacArthur, 1921), and dye-reduction gradients of another sand dollar, *Dendraster excentricus*, are like those of *Strongylocentrotus* but more distinct (Child, 1936a). The question whether the change in condition indicated by dye reduction in *Strongylocentrotus* and *Dendraster* occurs later in *Arbacia* and *Echinarachnius* remains open for further investigation.

In a long series of papers from 1914 on, Runnström has advanced a hypothesis of gradient pattern of sea-urchin egg and embryo in terms of two opposed overlapping gradients in the polar axis. These are regarded as concentration gradients of specifically different substances, the "animal" gradient decreasing in concentration basipetally, the "vegetal" gradient acropetally. Ventrodorsality is considered to be represented by another concentration gradient approximately at right angles to the polar gradients; and the lateral asymmetry of the larva, by still another. Since these are regarded as coexisting and interpenetrating, each must differ specifically from the others. His co-workers, particularly Hörstadius and Lindahl, have applied this hypothesis in interpretation of their experiments on echinoderm development.²⁷

Using potassium-free sea water with several species of sea urchins, Runnström (1925a) finds a differential susceptibility to lack of potassium

²⁷ See Runnström, Hörstadius, and Lindahl in Bibliography.

in the apicobasal and ventrodorsal axes, basal and ventral regions being most susceptible. According to his conclusions, water content and permeability to salts are highest in basal and ventral regions, and absence of potassium brings about loss of water, first from apical and dorsal regions of lower water content to the basal region and then from the whole egg or embryo; decrease in dispersion of colloids is also believed to occur. The differential susceptibility is regarded as resulting from differential permeability to potassium ions, decreasing from basal and ventral regions acropetally and dorsally (see Appendix V, p. 742). According to Runnström, the basal region is also more susceptible than the apical to high concentrations of sodium thiocyanate, Nile blue sulphate, and lithium salts; development in a CO-O₂ atmosphere in light shows injury in both apical and basal regions, and evidence of a ventrodorsal and left-right gradient is also found.²⁸

Most of Runnström's data on susceptibility are concerned with modifications of development rather than with cytolytic or death gradients. Differential modifications of development are considered in the following chapter, but his views require some further consideration here in connection with the more direct evidence concerning gradient pattern. He apparently regards the effects of external agents as more or less regionally specific, some acting on the animal, others on the vegetal gradient, and so on. It does not appear, however, that he has considered the possibility of differential tolerance to external agents and of differential recovery after temporary exposure. As will appear more clearly in the next chapter, these reactions of the organism may bring about differential modifications of form and proportion opposite in direction, as regards axial relations, to those resulting from direct action of the agent. Nor does the possibility that the gradient pattern or parts of it may undergo change during development seem to have been considered. There is often little evidence in his data of use of a wide range of concentrations and exposure periods. Whether, or to what extent, some of the modifications described represent differential inhibitions, differential tolerance, differential recovery, or regionally specific effects seems, at present, uncertain.

Differential cytolysis and death and differential dye reduction give no evidence of overlapping, specifically different gradients in the polar axis. They indicate a single polar gradient, the "animal" gradient in early stages, and, according to the recent studies of Child (1936a), a second "vegetal" gradient appearing later and partially obliterating or replacing

²⁸ Runnström, 1928a, b; 1929b; 1933; 1935a.

the original gradient. But, as noted in these studies, differential dye reduction gives no information as to presence or absence of regional specific differences. Oxidation-reduction reactions may differ as regards reacting substances, the reactions themselves, and the products formed but may still show the same rates or similar gradients of dye reduction. So far as the results of differential dye reduction and differential susceptibility are concerned, the specific overlapping gradients postulated by Runnström may or may not be present. On the other hand, concentration gradients of substance can accomplish nothing in the way of development without metabolism. Moreover, two opposed and overlapping substance gradients may be associated with a single gradient of rate or intensity. The overlapping polar gradients of yolk and protoplasm in many eggs and the difference in rate of cleavage and other developmental activities at different levels showing a single gradient decreasing basipetally will serve as an example. Specifically different substances may be present in apical and basal regions of the echinoderm egg at the beginning of development; if not present then, they doubtless are later. When present, they are undoubtedly concerned in differentiation of cells; but form and proportion—morphogenesis in its larger, more general aspects—appears, at least during earlier developmental stages, to depend to a much greater extent on quantitative factors of pattern, factors of rate or intensity, than on regional distribution of specific substances. Substance is significant in development only as it takes part in or affects activities; the activities, not the substances, are formative. Differential dye reduction indicates some of the activities and their quantitative differences and changes of rate but tells us nothing about the substances concerned. These differences and changes in rate appear to be essential factors in development of form and proportion, and they seem to the writer to offer less difficulty in interpretation of the more general features of early echinoderm morphogenesis than the specific concentration gradients of Runnström. Even if this is true, however, the specific concentration gradients may also be present. Nevertheless, the data at hand seem to favor the view that factors of rate or intensity are more important in determining form and proportions in the earlier stages of echinoderm development than specific or qualitative differences of substance. Definite patterns of form and proportion are evident before cellular and regional differentiations are distinguishable with certainty. Further investigation is, of course, necessary; but in spite of the fact that data and conclusions concerning different species are not in complete agreement as regards presence or appearance before gastrula-

tion of a secondary acropetal gradient, and in spite of the hypothesis of Ranzi and Falkenheim (p. 139) that there is no gradient, although their data indicate one, it appears evident that a gradient pattern of some sort is a fundamental factor in early echinoderm development. This will perhaps become even more evident in the following chapter. It remains for the future to determine to what extent use of different methods and procedures, of different species as material, and possibly preconceived views may be concerned in the lack of agreement and lack of information on certain points. In later chapters some analysis of other lines of recent experiment on echinoderm development will be attempted.

OTHER INVERTEBRATES

A few data are at hand concerning mollusks. Early developmental stages of several gasteropods show basipetal decrease of susceptibility, as indicated by cytolysis and death and later a local increase in the region which becomes the shell gland (Child, unpublished). Differential susceptibility to various agents has been observed in early cephalopod development (Ranzi, 1927; 1928; 1929*a, b*). In gasteropods and a cephalopod the apical region of the egg becomes more alkaline than the basal (Spek, 1934*a*; Ries und Gersch, 1936). Methylene blue leucobase (rongalite white) oxidizes most rapidly in the apical region of the egg of *Aplysia*, an opisthobranch gasteropod. The more rapid oxidation of the leucobase by single blastomeres, even by single or certain micromeres, observed by these authors, raises certain questions. Oxidation is more rapid in one cell of the two- and four-cell stages and in some of the micromeres of later stages. Since the prototroch of the early larva also oxidizes leucobase more rapidly than other cells, they regard the differential oxidation in earlier stages as indicating cell lineage of the prototroch and velum from one cell of the two-cell stage and a single quadrant of the four-cell stage and from certain micromeres of this quadrant. The cleavage is not followed in detail; and in the light of earlier studies on cell lineage in mollusks and annelids, such origin of the prototroch appears improbable. The possibility suggests itself that difference in condition associated with stage of the division cycle may be responsible for some of the differences in rate of leucobase oxidation in individual cells; such difference may also determine a difference in susceptibility to the toxic rongalite white, and the deep staining of certain blastomeres may result from injury. These authors also find that reduction of Janus green decreases in rate from the basal pole acropetally in maturation and cleavage stages of *Aplysia*.

Since the basal region of this egg consists largely of yolk, the apical region of cytoplasm, and the rate of cleavage and morphogenesis is much higher apically, this result is rather surprising. There can be little doubt that it, like the reduction differential observed by Gersch in *Paramecium* (p. 92, footnote 2), results from injury by the very toxic Janus green to the apical region, and consequent retardation or absence of reduction there. There is no evidence in this work of any attempt to determine whether different concentrations of dye and different staining periods would give different results.²⁹

Direct evidence of physiological differentials or regional specificities in early stages of arthropod development appears to be lacking, except for demonstration of differential susceptibility to cyanide in the insect *Bruchus quadrimaculatus* (Coleoptera). In stages from egg-laying to 6½ hours susceptibility becomes highest on the ventral surface, and the most susceptible area gradually becomes localized in the median ventral, presumptive prothoracic-maxillary region. From 6½ to 12 hours ectodermal susceptibility decreases from this region anteriorly through the head region and posteriorly through the embryonic plate; from 12 to 16 hours it remains essentially the same. The locus of highest susceptibility in the ventral prothoracic region is the position of the "differentiation center" of Seidel (pp. 515-21). The aggregations of nuclei at certain regions of the cortical layer of the insect egg, their changes of position in some eggs, and the progress of formation of cell boundaries about them from certain egg regions indicate, of course, presence of physiological pattern of some sort; but of what sort, these observations do not show.

During the summer and autumn, 1940, suggestive data on differential reduction of methylene blue and Janus green in the ovaries of the insect *Drosophila hydei* have been obtained (Child, unpublished). The ovaries consist of parallel series or strings of follicles in each of which the basal cell becomes the egg, the other cells becoming accessory or nurse cells. The apical pole of the egg is toward the apical ovarian pole, that is, the pole where follicle development begins; and the ventral side of the egg is toward the outer ovarian surface. If overstaining and differential injury are avoided, dye reduction in low oxygen progresses basipetally in the

²⁹ These authors have investigated various aspects of cytoplasmic differentials and differentiations in eggs and early stages of a considerable number of animals, with certain results of interest and undoubted value; but in some respects the data presented appear inadequate as a basis for the conclusions drawn and suggest the need of a considerably wider range of experiments. See Ries und Gersch, 1937; Ries, 1937; Gersch und Ries, 1937; and their citations of earlier papers.

apical ovarian regions consisting of the younger follicles and from the outer ovarian surface inward. In older single follicles in which the growing oöcyte is distinguishable from nurse cells in living material, the nurse cells reduce before the oöcyte, and reduction in the oöcyte and in the follicle wall progresses basipetally. In the full-grown egg cell a basipetal reduction gradient usually appears, and some evidence of a ventrodorsal gradient has been obtained; but this gradient, if present, is slight. Since the data show a uniform and constant relation between morphological and physiological pattern of ovary, follicle, and egg, they suggest that the ovarian differential determines which cell in the follicle becomes egg and the axiate pattern of that egg.

EARLY CHORDATE DEVELOPMENT

ASCIDIANS

A polar-dorsiventral pattern is directly visible in some ascidians in differences in color and appearance of certain cytoplasmic regions, but this pattern appears only after fertilization. Moreover, the visible pattern is of no significance for ascidian development, for in other species no such pattern is visible, but development follows the same course, and a polar-dorsiventral pattern is inferred.³⁰ But it is perhaps worth repeating that, whatever the egg pattern is, it is not necessary for ascidian development. Ascidians can develop from pieces of the body, from stolon tips and isolated pieces of stolon, from buds, and from aggregations of cells. Evidently, the regional cytoplasmic pattern of the ascidian egg is a product or expression of a more fundamental pattern of some sort; that is, more or less regional differentiation occurs in this egg before cleavage begins. Apparently, the only direct evidence of gradient pattern in ascidian development is the differential reduction of potassium permanganate in *Corella willmeriana* (Child, 1927*d*). In early cleavage the reduction gradient is distinctly basipetal (Fig. 50, *A*). In the gastrula a region corresponding to the dorsal region of the amphibian embryo (see Fig. 156) in its relation to the blastopore, though not in relation to the primary polarity of the egg, reduces most rapidly, with lateral margins of the blastopore following (Fig. 50, *B*, *C*). Even before outgrowth of the tail, increase in rate of reduction appears in the region of the prospective tail (Fig. 50, *D*), and the longitudinal gradient becomes two-armed or U-shaped; that is, a new gradient, opposed in direction to the primary

³⁰ Conklin, 1905*a*, *b*, *c*, 1906, 1931; Dalcq, 1935, 1938; and various citations by these authors.

gradient, has appeared, as apparently in annelids and also in echinoderms. With outgrowth of the tail a very distinct reduction gradient appears in it, the tip being the most rapidly reducing region of the larva; the neural

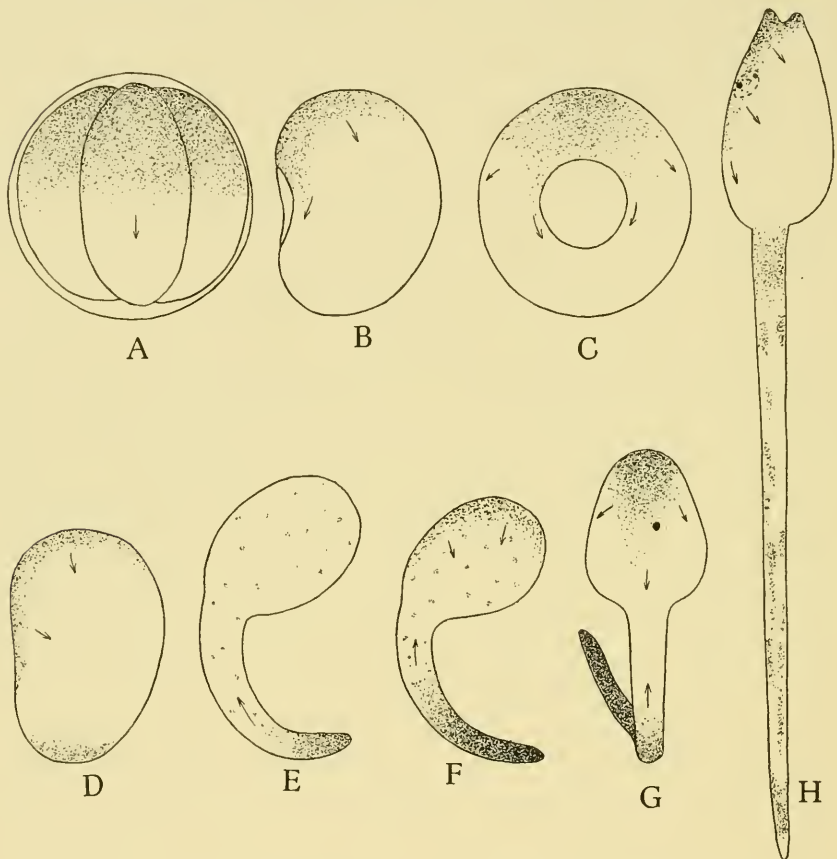


FIG. 50, A-H.—Differential KMnO_4 reduction in larval development of the ascidian *Corella willmeriana*, arrows indicating direction of progress. A, four-cell stage with basipetal decrease in rate; B, C, gastrula in lateral and dorsal view; D, increase in rate of reduction in region of future tail; E, caudal gradient with higher rate of reduction at tip than in any other part of body and early reduction of scattered tunic cells; F, G, later stages of larval development; H, caudal resorption about to begin (after Child, 1927d).

side of the larval body and the end reduce more rapidly than the other side (Fig. 50, E, F, G). During its development the tail is also the most susceptible region of the larva. In the full-grown larva the caudal reduction gradient is still distinct; but, as compared with the body, rate of

caudal reduction is relatively less than earlier. Before resorption of the tail begins, its reduction gradient becomes very slight; during resorption traces of it may persist, or only irregular areas of more rapid reduction scattered along the tail appear (Fig. 50, *H*). As this figure indicates, the more rapid reduction on the neural side of the larval body persists.

The appearance at the gastrula stage of a region of high rate of reduction corresponding to the dorsal inductor region of amphibia, the dorsal lip of the blastopore, is of interest, as compared with the higher rate of respiration and oxidation in this region of the amphibian gastrula observed or indicated by most experimental data (pp. 153-58) and in relation to the view, now widely held, that there are fundamental similarities in spatial embryonic pattern throughout the chordates. Differential permanganate reduction does not, of course, give any information as regards presence or absence of regional cytoplasmic specificities. It does suggest, however, that a quantitative gradient pattern perhaps underlies and precedes the regionally specific pattern.

CYCLOSTOMES

Differential susceptibility to ammonia and acetic acid of the lamprey (*Entosphenus appendix*), as determined by Hyman (1926*b*), is shown in Figure 51. The susceptibility gradient of the unfertilized egg (*A*) and early cleavage stages (*B*, *C*) is basipetal. In more advanced cleavage it is in general basipetal; but some irregularities as regards cells or cell groups appear, perhaps because susceptibility differs at different stages of the division cycle (*D*). In the early blastula the basipetal gradient is distinct and symmetrical (*E*); but in the later blastula one side, presumably dorsal, becomes more susceptible than the other (*F*, *G*). The region of invagination is more susceptible than any other part in the early gastrula (*H*), the dorsal side is more susceptible than the ventral (*I*, *J*), and this dorsiventral difference increases as gastrulation proceeds (*K*, *L*). As the neural groove develops, its anterior region becomes the most susceptible part of the embryo (*M*), but the original basipetal (anteroposterior) gradient and the higher susceptibility of the blastopore region are still evident in the later stages of disintegration (*N*). With elongation of the head region death progresses from the anterior end of the brain posteriorly in the dorsal region and laterally and ventrally from this region, and a region extending dorsally and anteriorly from the blastopore also shows high susceptibility (*O*, *P*); this, according to Hyman, is the segmental plate or somite-forming region. At the time of hatching, the gradient

is predominantly anteroposterior (*Q, R*); 2-3 days later susceptibility of the posterior region has increased so that anterior and posterior arms of the gradient are almost equal (*S, T*). No essential difference in the re-

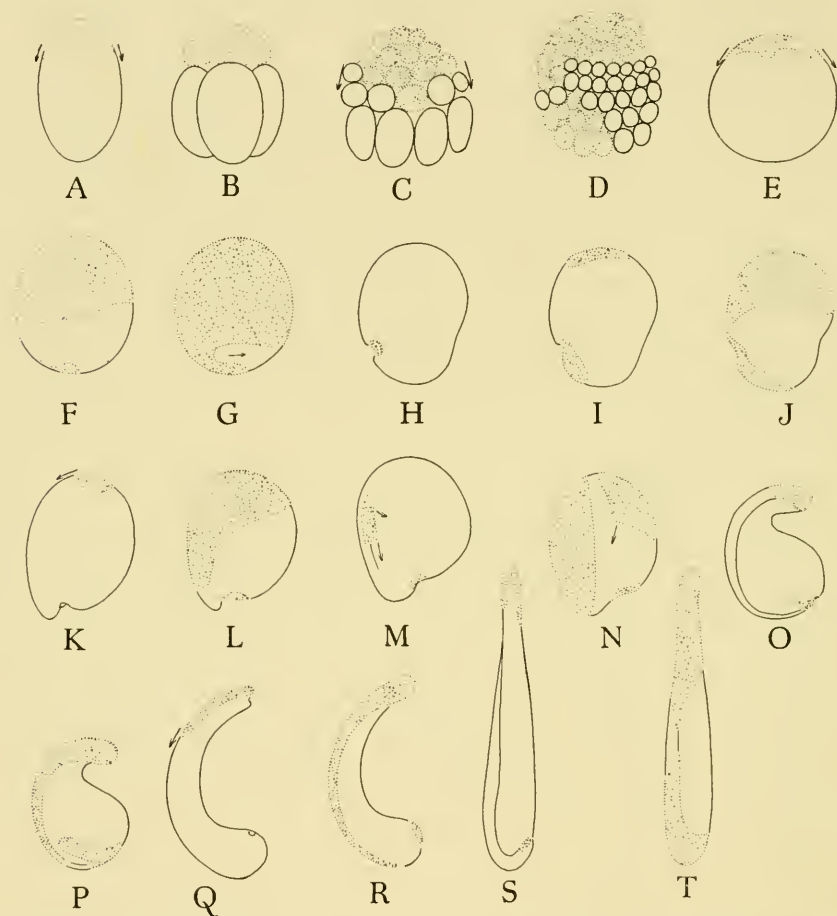


FIG. 51, A-T.—Differential susceptibility in development of the lamprey *Entosphenus appendix*; disintegrated regions indicated by dotted bounding-lines; and the chief directions of progress of disintegration, by arrows. *A*, unfertilized egg; *B*, eight-cell stage; *C*, about thirty-two cells; *D*, later cleavage; *E*, late cleavage or early blastula; *F, G*, two stages of disintegration in late blastula; *H-J*, three stages of disintegration at beginning of gastrulation; *K, L*, later gastrula; *M, N*, neural groove stage, disintegration progressing posteriorly, but original apicobasal gradient evident in later disintegration (*N*); *O, P*, susceptibility decreasing posteriorly from the elongating head along the nervous system and an increased susceptibility along the segmental plate; *Q, R*, at hatching, susceptibility decreasing posteriorly along the nervous system, with secondary region of high susceptibility posteriorly; *S, T*, 2-3 days after hatching, anterior and posterior arms of the gradient almost equal (after Hyman, 1926b).

gional susceptibilities to the two agents was observed; the same pattern appears with both. The relation between susceptibility and developmental activity seems evident. The higher susceptibility of the presumptive dorsal side, and later of the actual dorsal side and the region about the blastopore, indicates a change in condition of this region as gastrulation approaches. This is of interest in comparison with the increase in rate of reduction in the region corresponding to the dorsal lip of the blastopore in the ascidian (Fig. 50, *B*, *C*) and the increase in susceptibility of the amphibian dorsal region at gastrulation; it is also in line with the evidence of higher rate of respiration and of dye reduction in this region (see pp. 153-58).

TELEOSTS

Differential susceptibility of developmental stages of three teleost fishes, *Tautogolabrus adspersus*, *Fundulus heteroclitus*, and *Gadus morrhua*, has been determined by Hyman (1921). Figure 52 shows some of the stages. During early cleavage of *Tautogolabrus* susceptibility decreases from center to periphery of the blastoderm (*A*). In the later blastoderm the center is no longer the most susceptible region; disintegration begins at the posterior border of the blastoderm, that is, in the region which is to become the embryonic area, and proceeds anteriorly (*B*, *C*). This region represents the dorsal lip of the blastopore, and its high susceptibility at this stage corresponds with the findings in the ascidian, cyclostome, and amphibian. After the embryo appears, the gradient in it is from anterior to posterior and from dorsal to ventral (*D*, *E*). In still later stages the posterior end of the embryo becomes a secondary region of high susceptibility, and death progresses anteriorly from it, as well as posteriorly from the head region and laterally and ventrally from the dorsal region (*F*). In its earlier stages *Fundulus* resembles *Tautogolabrus*, but in the embryo the posterior region of high susceptibility appears earlier and, up to advanced embryonic stages, is more susceptible than the anterior end (*G-I*).

The development of *Gadus* shows somewhat different susceptibility relations in earlier stages (Fig. 52, *J-P*). In this species the germ ring develops early, and it and the embryonic shield are much more distinct and more sharply marked off from other parts than in the other two forms. In the earliest stages observed, the periphery of the blastoderm where the germ ring is forming is more susceptible than the center (*J*). In early germ-ring stages one side of the ring is more susceptible than the other; and when the embryonic shield forms, it is the most susceptible region (*K*, *L*). In more advanced stages of the shield and in early stages of the

embryo an anteroposterior gradient appears, corresponding to the polar axis of the embryo (M , N). At a somewhat later stage, before closure of the germ ring, a second region of high susceptibility appears at the posterior end of the embryonic shield and persists in the posterior end of the embryo (O , P). As particular organs—the eyes, the heart, etc.—are local-

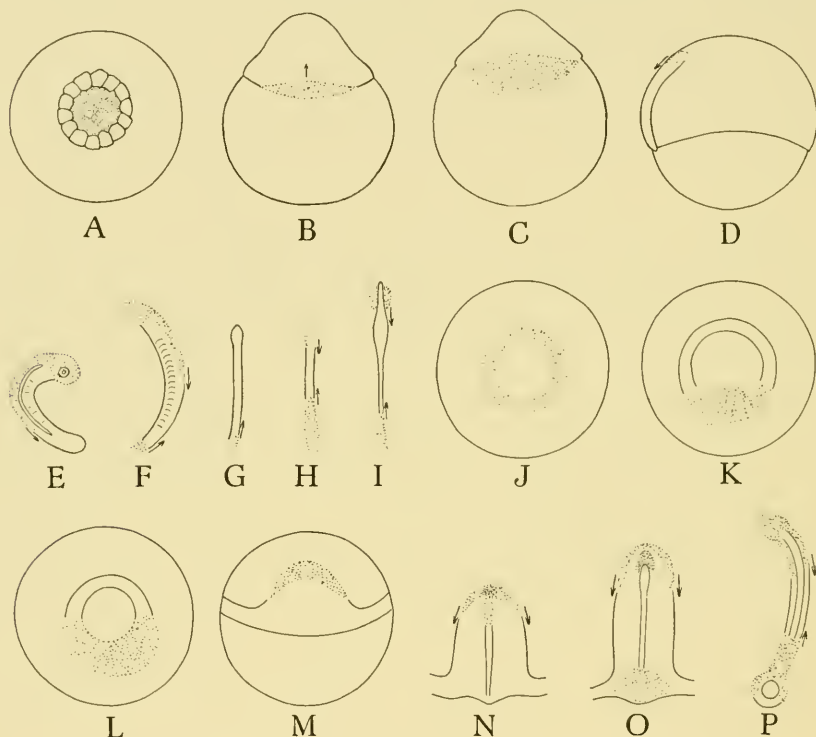


FIG. 52, A-P.—Differential susceptibility in early teleost development; regions and progress of disintegration indicated as in Fig. 51. A-F, *Tautoglabrus adspersus*; G-I, *Fundulus heteroclitus*; J-P, *Gadus morrhua* (after Hyman, 1921).

ized and develop, they appear as regions of high susceptibility; in the embryonic heart susceptibility decreases from the venous end.

It is suggested by Hyman that the decrease of susceptibility from the center of the early blastoderm peripherally in *Tautoglabrus* (Fig. 52, A) indicates a more primitive type of development with developmental activity centered about the animal pole; the early appearance of higher susceptibility peripherally in the blastoderm of *Gadus* is regarded as associated with the earlier appearance and more definite development of the germ ring in this species. In any case, the relation of differential sus-

ceptibility to developmental activity is evident in these teleosts, as elsewhere. In Figure 52, *B*, *C*, *K*, and *L*, the region representing the dorsal lip of the blastopore shows high susceptibility; in *B* and *C* stages are figured showing the highest susceptibility in the region of early invagination. During elongation of the embryonic shield its anterior end becomes the most susceptible region; but sooner or later, at somewhat different stages in the different species, a second region of high susceptibility appears posteriorly, as in other segmented animals.

An electric-potential difference exists between apical and basal poles in early stages of *Fundulus* development and undergoes cyclical reversal, apparently in relation to cyclical changes in the cells. A potential difference also occurs along one axis of the blastoderm (Hyde, 1904). At the time of maturation alkaline colloids accumulate apically, the blastodisc becoming distinctly marked off from the acid remainder (Spek, 1933).

AMPHIBIA

The great volume of investigation concerned with amphibian development in recent years has revealed many facts and perhaps presented even more problems. We have learned something about the pattern of amphibian development, but the problem of pattern remains and continually presents new aspects. Some of these are touched upon in later chapters; the present concern is chiefly with some of the more direct evidence bearing on the general characteristics of amphibian spatial or regional pattern in earlier stages of development.

The protoplasm-yolk gradient of the amphibian egg, the superficial pigmentation present in most species except in the basal region of high yolk content, and the gradient, decreasing basipetally, in rate of cleavage indicate an apicobasal pattern. Dorsiventrality is clearly indicated in the fertilized eggs of some anurans by the gray crescent, a less deeply pigmented, subequatorial region between the deeply pigmented apical, and the light basal region, decreasing in width laterally and ventrally from the mid-dorsal region. It is indicated in Figure 53, *A-C*. In eggs of other amphibia a corresponding region is more or less clearly indicated by a difference in appearance from other parts. Observations on differential susceptibility, as indicated by cytolysis, give evidence of a definite pattern of physiological condition undergoing progressive change with the progress of development.³¹

³¹ Bellamy, 1919; Bellamy and Child, 1924; material, *Rana pipiens*, *Chorophilus nigratus*, *Bufo americanus*, and some data on *Amblystoma tigrinum*.

In the unsegmented egg disintegration progresses basipetally from the apical pole but more rapidly in a broad band over the dorsal surface to the region of the gray crescent (Fig. 53, *A*). In early cleavage essentially

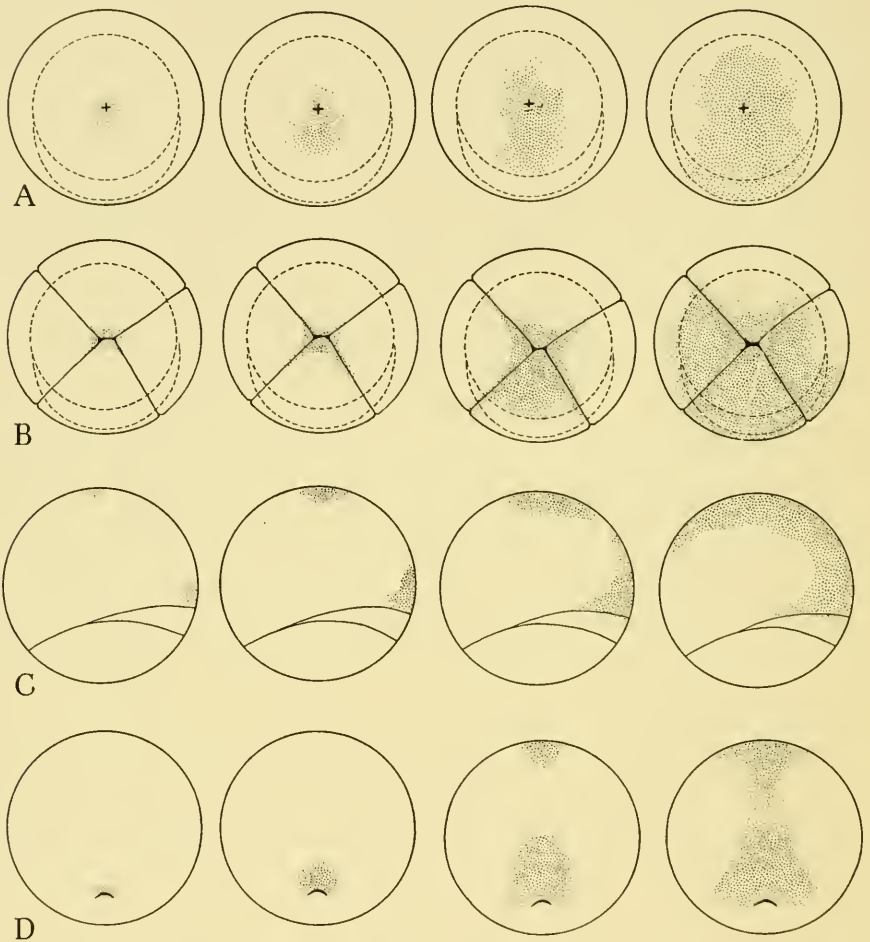


FIG. 53, *A-D*.—Differential susceptibility in early development of *Rana pipiens*. *A*, four stages of disintegration in undivided egg; *B*, four stages in early cleavage; *C*, in late blastula; *D*, in early gastrula (from Bellamy, 1919).

the same susceptibility pattern appears (Fig. 53, *B*). In late cleavage a region near the middle of the gray crescent begins to disintegrate at about the same time as the apical pole, and the two areas spread and meet along the dorsal side of the embryo (Fig. 53, *C*) and gradually extend basipetally

and ventrally over other parts. The region of the dorsal lip of the blastopore is more susceptible than the apical pole in the early gastrula; from this region disintegration extends anteriorly to a greater extent than in earlier stages, but also extends basipetally from the apical pole (Fig. 53, *D*).

The anterior end of the embryo is localized near, or more or less ventral to, the apical or animal pole of the egg, according to the species; the apical region, then, becomes anterior. After gastrulation is completed, two areas of high susceptibility still persist, one anterior, the other dorsal; but the dorsal area is less susceptible than the anterior. In the earlier neurula stages disintegration begins in the median anterior region of the neural plate; and in slightly later stages, usually at two points where the optic primordia are developing. From this anterior region it spreads posteriorly along the neural plate and joins the area of disintegration at the posterior end of the embryo. Susceptibility of neural-plate stage to radium shows essentially the same differentials (Stachowitz, 1914). In early stages of organ development optic vesicles, nasal pits, ventral suckers, and tail bud all appear as local areas of high susceptibility with local radial or axial gradients.

The data on susceptibility indicate that the dorsal region is different in some way from lateral and ventral regions, but the apical region is primarily the most susceptible. As development progresses, the relative susceptibility of the region which is to become the dorsal lip of the blastopore increases, until at the beginning of gastrulation it is the most susceptible part of the embryo, and in it susceptibility decreases anteriorly and laterally from the blastopore. This is the region which, after invagination, becomes the so-called "organizer" or "organization center" (see pp. 454-70). In a study of differential susceptibility in other amphibian species Cannon (1923) found practically no uniformity in the susceptibility of different regions at various stages of development (see Appendix VI, p. 743).

Recent investigations on the respiration of the dorsal lip region, as compared with other parts, are of much interest in relation to the role of this region as inductor of the neural plate and to the data on susceptibility. In thirty-six out of forty-four cases (82 per cent) in which the dorsal lip region was destroyed, oxygen uptake was found to be lowered by 30 per cent or more, as compared with controls in which an equivalent destruction of an indifferent region had been accomplished. Gastrulae from which the dorsal lip region has been removed have a lower CO_2 production than those from which an equal volume of cells has been re-

moved from the ventral marginal zone of the blastopore. Also, the CO_2 production of the isolated dorsal lip region is distinctly higher than that of an equal cell volume from the ventral marginal zone (J. Brachet, 1934a, b). Later determinations by Waddington, Needham, and Brachet (1936) on fragments taken immediately above the dorsal lip and fragments of presumptive ventral ectoderm showed oxygen uptake of the same order of magnitude in both. According to determinations by J. Brachet (1936), however, CO_2 production is about 85 per cent higher in pieces from the dorsal lip than in ventral pieces; oxygen uptake per milligram of nitrogen is 35 to 40 per cent higher in the dorsal than in the ventral pieces; and the respiratory quotient is 1.06 in the dorsal, as compared with 0.76 in ventral pieces. The higher CO_2 production in the dorsal lip results in part from a higher absolute rate of metabolism and in part from the higher respiratory quotient.

Simultaneous determination of oxygen uptake of the two sides of single intact eggs and embryos showed the dorsal side averaging 47 per cent higher than the ventral in gastrulae of the frog (*Rana sylvatica*).³² With orientation of the embryos in the capillary so that the dorsal lip was symmetrically placed in relation to the two sides of the apparatus, no significant difference in oxygen uptake appeared. The authors suggest that the earlier failure by Waddington, Needham, and Brachet (1936) to discover a difference in respiration of dorsal and ventral piece probably resulted from use of pieces taken from above the dorsal lip rather than from the dorsal lip itself. They also point out that alteration of respiratory rate after isolation may be concerned in determinations on isolated pieces. In unfertilized eggs of *R. pipiens* they found no consistent difference in oxygen uptake between apical and basal hemispheres. If there is any actual difference, it is probably very slight until activation occurs on fertilization or by other means, and it may decrease when the egg remains for some time without fertilization.

Determinations of oxygen uptake, respiratory quotient, ammonia production, anaerobic and aerobic glycolysis in very small fragments of dorsal and ventral regions of amphibian embryos of various species with the Cartesian diver used as an ultramicrorespirometer show no consistent differences in oxygen uptake, a higher respiratory quotient dorsally (about 1), as found by Brachet, anaerobic glycolysis of dorsal pieces about three

³² J. Brachet and Shapiro, 1938. Eggs and embryos were introduced singly into capillary tubes with lumen about equal to, or slightly larger than, their diameter, and each end of the tube was connected with a manometer.

times that of ventral, ammonia production higher dorsally, and little or no aerobic glycolysis in any part.³³ It is not evident that the very small fragments used in these determinations contribute in any way to greater accuracy or certainty of results. If metabolism is altered by isolation, greater alteration may be expected with smaller than with larger pieces; in isolated pieces of planarians and hydroids such differences appear. The data do not indicate whether isolation alters metabolism in these embryonic pieces or whether size of piece may be a factor in rate after isolation.

According to another series of determinations of oxygen uptake,³⁴ that of the apical half of the blastula is 3.1 times that of the basal half, that of the dorsal inductor region twice that of the intact gastrula, that of the neural plate of the neural stage 2.61 times that of the whole neurula, and that of the anterior region of the later embryo higher than that of the posterior part.

In a later paper J. Brachet (1939) finds little difference in oxygen uptake between dorsal and ventral sides in *R. fusca* at the beginning of gastrulation and agrees with Boell, Needham, *et al.* that the great difference found earlier probably resulted from the presence of two cell layers in the dorsal region; that is, the determinations on single embryos required so much time and temperature was so high that invagination was advanced before they were completed. However, it is perhaps still a question whether the second cell layer beneath some part of the dorsal ectoderm would increase oxygen uptake 47 per cent. There is also the possibility the oxygen uptake increases in the invaginated portion of the dorsal region. Brachet's later determinations of CO₂ production by a colorimetric method show the dorsal side 29 per cent higher than the ventral; but this difference is, at least in part, due to the difference in respiratory quotient. He also finds dorsal pieces more susceptible than ventral to agents which inhibit glycolysis (monoiodoacetate and NaF). Carbohydrate metabolism, indicated for the dorsal region by the respiratory quotient about unity, he regards as not essential to induction, and he shows that induction is not necessarily prevented by inhibition of glycolysis.

³³ Needham and Boell, 1938; Boell, 1938; Boell, Needham, and Rogers, 1939; Boell and Needham, 1939; Boell, Koch, and Needham, 1939; Needham, Rogers, and Shih-Chang Shen, 1939. More recently Boell and Nicholas (1940, *Abstr. Amer. Soc. Zool., Anat. Rec.*, 78, 4, Suppl.) have found a basipetal decrease in respiration dorsally and a steeper decrease ventrally, but they maintain that when corrections are made for the different amounts of yolk in the different regions actual quantitative respiratory differences do not appear. See also Needham, 1939, "Biochemical aspects of organizer's phenomena," *Growth*, Suppl.

³⁴ Fischer und Hartwig, 1938; oxygen as mm³O₂ per 10 mg. dry weight per hour.

Still another series of determinations of oxygen uptake in pieces from *Amblystoma* and *Rana* embryos³⁵ shows the dorsal lip region much higher than the ventral lip and slightly higher than the presumptive epidermis, but Barth points out that the cells of the dorsal lip contain more yolk than those of the epidermis; consequently, intensity of respiration in the metabolizing protoplasm of the dorsal lip must be considerably higher than appears from the data. On the ventral side oxygen uptake decreases basipetally from the apical region.

Somewhat earlier, glycogen distribution in amphibian embryos was investigated by Woerdeman (1933*a, b, d*). His findings show more or less uniform distribution in the apical hemisphere with decrease in the dorsal lip on invagination. Similar conclusions were reached by Tanaka (1934), and a decrease was also observed on invagination of transplanted pieces of the dorsal lip region (Raven, 1933*a, 1935a*). Using a different method for demonstrating glycogen, Pasteels (1936*c*) maintains that the supposed decrease does not occur. However, work with a quantitative microchemical method indicates that before gastrulation glycogen decreases basipetally from the apical pole and that during gastrulation there is decrease in amount in all regions, but that decrease is greatest in the invaginating material (Heatley and Lindahl, 1937). These observations are interesting in relation to the data of Boell, Needham, *et al.* on glycolysis.

Most amphibian eggs and embryos are unsatisfactory material for dye reduction because of their pigmentation. Recently, however, some observations have been made on unpigmented or slightly pigmented forms. Staining the unpigmented neurula of the urodele *Triton cristatus* by re-oxidation of the leucobase of brilliant cresyl blue and observing reduction under strictly anaerobic conditions, Fischer und Hartwig (1936) found reduction most rapid in the floor of the open neural plate. Using the same material and the same procedure, Piepho (1938) followed the course of reduction in blastulae and gastrulae. In blastulae reduction occurred most rapidly in a region extending from the apical pole almost to the equator and from there progressively to the basal pole. Gastrulae, so far as they gave definite results (60 per cent of the total), showed, like blastulae, more rapid reduction in the region apical to the equator and also in the region of the dorsal lip of the blastopore, the inductor region. Whether one of these regions reduced more rapidly than the other could not be determined. In these experiments reduction occurred under nitrogen, that

³⁵ Barth, 1939*a, b*; oxygen uptake in cubic millimeters of oxygen per 100 mg. dry weight per hour.

is, oxygen was completely excluded from the beginning. The lesser differences in rate are undoubtedly more clearly distinguishable with gradual oxygen decrease than with complete exclusion at once. Probably with gradual decrease of oxygen a reduction gradient progressing basipetally from the apical pole would have appeared instead of reduction of the apical hemisphere without differential.

In eggs and embryos of *Triturus rivularis* there is considerable variation in depth of pigmentation. Some lots of eggs were found by Child to be light enough to permit observation of reduction.³⁶ With gradual decrease of oxygen dye reduction could be observed to progress basipetally in blastulae, but whether a dorsiventral differential was present could not be determined; if present, it was very slight. In the naked early gastrula, however, reduction was most rapid at the dorsal border of the blastopore and progressed laterally and anteriorly from this region. Apical and dorsal reduction could not be observed in the same individual because of the flattening, but embryos in proper orientation showed reduction decreasing in rate from the apical region basipetally. In stages with open neural plate rate of reduction appears highest in the floor of the plate and decreases from the anterior region posteriorly. It occurs over the whole plate, including a narrow region just outside the neural folds, before reduction is clearly evident in the general ectoderm.³⁷ A few observations on elongated embryos with closed neural folds showed rate of reduction decreasing from the head posteriorly along the dorsal region and laterally and ventrally from that, with a posterior region of higher rate as development of the tail began.

Using the method of Giroud and Bulliard (1933) for indicating occurrence and distribution of sulphydryl compounds by a color reaction, J. Brachet (1938) has shown a definite and changing distribution during development of several amphibians of what he believes to be sulphydryl-proteins. In the unfertilized egg and soon after fertilization the reaction occurs in a region about the apical pole. Some 2 hours later it is localized more or less closely in a crescent, corresponding, in forms in which dorsal and ventral sides are distinguishable, to the position of the gray crescent of the frog's egg. In later cleavage and blastula stages the reaction occurs

³⁶ Slight staining with Janus green of blastulae within the membrane was found possible after removal of the jelly. With care the membrane can be removed from gastrulae; but, when naked, the gastrulae become flattened on the surface in contact.

³⁷ The *Triturus* material was provided through the kindness of Dr. V. C. Twitty, and his skill in removing the membranes of early gastrulae was of great assistance.

in the apical half, sometimes extending farther basipetally on one side. At the beginning of gastrulation the apical region still reacts strongly, the reaction extends farther basipetally on the dorsal than on the ventral side, and the dorsal lip of the blastopore shows in most cases an intensification of the reaction. In the neurula the reaction is most intense anteriorly on the floor of the neural plate and about the blastopore, less intense along the whole dorsal region. The head in later stages reacts most intensely, but the reaction occurs along the whole dorsal region. These data are of special interest in relation to the widely accepted view that sulphhydryl compounds are essential factors in oxidation-reduction. It is perhaps also of some significance that, except for stages just before and during early cleavage, the regions of most intense reaction are practically identical with those of highest susceptibility and most rapid dye reduction.

Although the different lines of evidence are not in complete agreement on certain points, they all show the presence of a definite spatial pattern of physiological condition and activity. This pattern is on a molar or regional scale, with gradation in condition from the apical pole basipetally and from the dorsal region ventrally. A progressive change in physiological condition and apparently, according to most of the data, a greater increase in rate of metabolism of some kind occurs in the dorsal region than elsewhere in the course of pregastrular development. Most of the available evidence indicates that at the time of gastrulation the region which becomes the dorsal lip of the blastopore, and after invagination the inductor also, becomes the most intensely active region of the embryo. The higher respiratory quotient of this region, indicating that carbohydrate metabolism is predominant, has already been noted. Since the respiratory quotient of the whole embryo later approaches or becomes unity, it is conceivable that the dorsal region, in consequence of its higher level of activity, exercises a certain degree of dominance over other parts in raising their metabolic levels; and with the rise in level, change in the character of metabolism may perhaps occur. In fact, it is suggested by Waddington, Needham, and Brachet (1936) that the change in metabolism indicated by the increase in respiratory quotient may spread over the embryo from the dorsal lip. The question whether the high metabolic level of the dorsal region is concerned in its action in inducing development of the neural plate after its invagination is also of fundamental importance, but its consideration is postponed to a later chapter, in which the problem of induction in general is taken up.

THE CHICK EMBRYO

Determinations of oxygen uptake by Shearer (1924) showed a higher rate in anterior than in posterior parts; but in a later paper, without further experiment, the earlier data are discarded as without significance

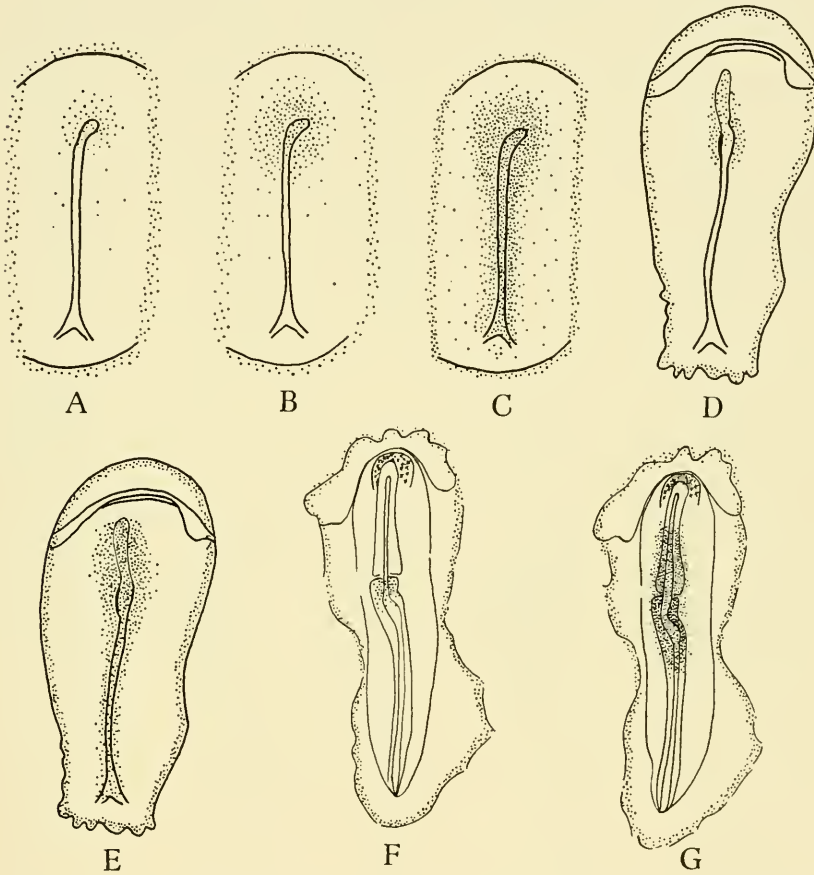


FIG. 54, A-G.—Differential reduction of Janus green in earlier stages of the chick embryo; reduction indicated by stippling. A-C, progress of reduction in primitive-streak stage; D, E, head-process stage; F, G, stage of first intersomitic groove; injury of neural folds and retardation of reduction indicated by small crosses (from Rulon, 1935).

(Shearer, 1930). A recent study of dye reduction has given results of value (Rulon, 1935). Reduction of Janus green to the red form by the embryos occurs readily in low oxygen. In primitive-streak and head-process stages rate of reduction decreases posteriorly from the region of the node (Fig. 54, A-C) and the head process (Fig. 54, D) and laterally from

the median region. The margin of the area pellucida where yolk is being digested also reduces rapidly (Fig. 54, *A-E*). At the stage of the first somitic groove the border of the anterior end of the neural plate is just beginning to fold dorsally and is so highly susceptible to the dye that, when the rest of the embryo is appreciably stained, it is irreversibly injured, and reduction in it is greatly retarded or does not occur at all; with slight staining, however, it shows a very high rate of reduction. In Figure 54, *F* and *G*, this region is indicated by small crosses as injured. At this stage the region of the node reduces somewhat less rapidly than the anterior region, and reduction progresses anteriorly and posteriorly from it (Fig. 54, *F, G*). At stages of three to four somites the region of the neural folds most susceptible to the dye is slightly posterior to the anterior end (Fig. 55, *A*); as in the earlier stage, it is a region of very rapid reduction after slight staining. Except for the neural folds, the region of the node reduces most rapidly with rate decreasing anteriorly and posteriorly from it (Fig. 55, *A-C*). Somewhat more slowly reduction occurs at the extreme anterior end of the head and progresses posteriorly, except for the injured neural folds, which reduce slowly or not at all. At the stage of eleven to twelve somites reduction is first evident anteriorly in the open neural folds of the anterior head region, the region of the neuropore, and the lateral regions, which are to form the optic vesicles, reducing most rapidly in this anterior region (Fig. 55, *D, E*). These regions also stain somewhat more deeply than other parts of the neural plate. Rate of reduction decreases posteriorly from the anterior end over most of the embryo; but the primitive streak, still present in the posterior region, appears as a second region of rapid reduction, with rate decreasing anteriorly and posteriorly from it, and the developing heart now becomes a region of rapid reduction (Fig. 55, *D, E*). Preceding torsion of the embryo, the level at which torsion occurs is temporarily a region of rapid reduction. At the twenty-somite stage torsion is completed, and flexion of the head is taking place. The regions of most rapid reduction at this stage are the primitive streak, the auditory vesicles, the future gill slits, and the region of the hindbrain, where flexion is greatest. The reduction gradient is now predominantly from the anterior end (Fig. 55, *G, H, I*); reduction in the primitive streak is relatively less rapid than earlier, and the gradient does not extend very far anteriorly from it. The region of most rapid reduction posteriorly is the future tail bud (Fig. 55, *G, H*). Figure 55, *J*, shows reduction in an early stage of the limb buds. Even before

any marked outgrowth the limb buds are regions of rapid reduction with decrease in rate more or less radially from a center, as in buds generally. In these embryos the rapid reduction in the region of the heart and gills

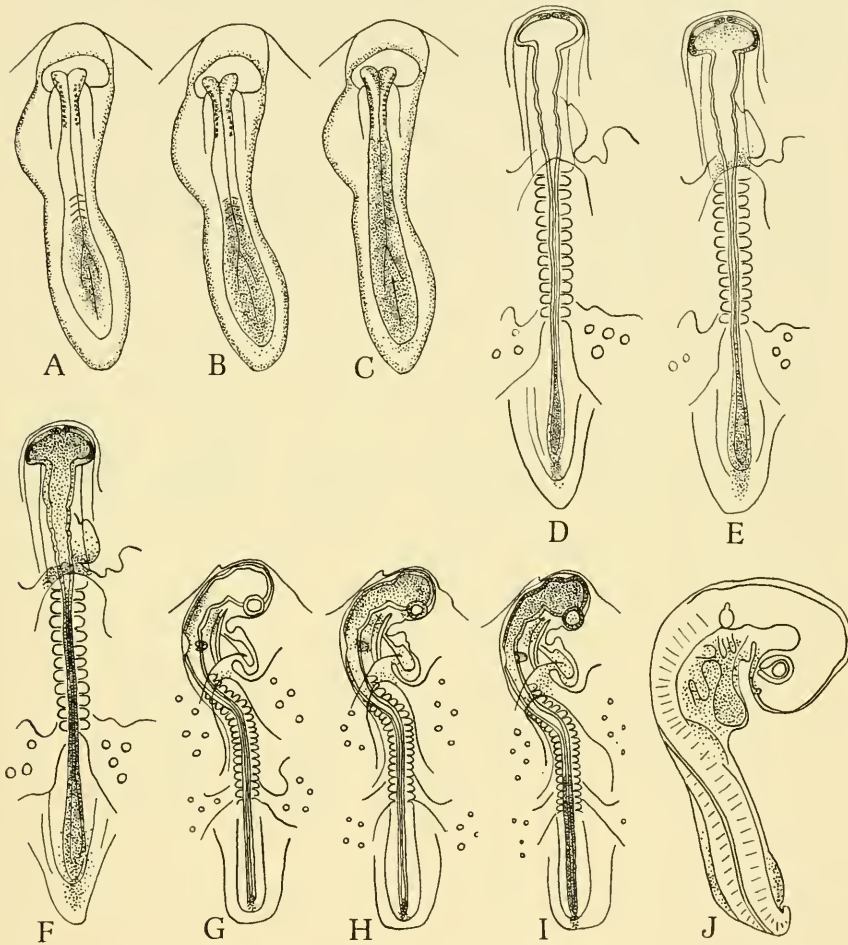


FIG. 55, A-J.—Differential reduction of Janus green in later stages of chick embryo. A-C, four-somite stage with injury and retardation or absence of reduction in neural folds; D-F, twelve-somite stage; G-I, twenty-somite stage; J, early limb-bud stage (from Rulon, 1935).

may be in part due to more rapid decrease of oxygen there in consequence of the crowding of parts. In still later stages each feather germ stands out as a region of more rapid reduction than the skin about it.

The reduction gradient in three stages of the developing heart is shown in Figure 56, A-C. The posterior region, where the omphalomesenteric veins fuse, shows the highest rate with progressive decrease anteriorly, but with higher rate on the right side (left in the figures).

Studies of differential susceptibility to cyanide, ammonia, and sodium hydroxide by Hyman (1927*a*), to ultra-violet radiation by Hinrichs (1927),

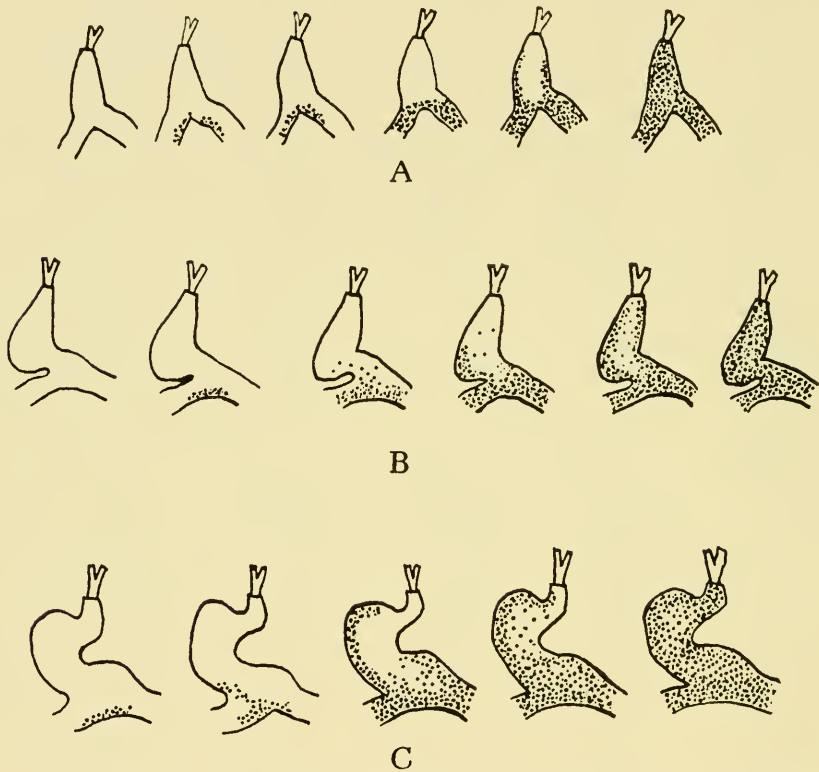


FIG. 56, A-C.—Differential dye reduction in three developmental stages of chick heart (from Rulon, 1935).

and to hydrocyanic acid by Buchanan (1926*c*) are in essential agreement, not only with each other but with the results of differential dye reduction, so far as the same stages are concerned. In primitive-streak and head-process stages a simple anteroposterior gradient appears. From early neural fold stages on, the anterior region and the region of the node are, in general, most susceptible; but other regions of high susceptibility appear, some temporarily. In early somite stages the region of closure of the

neural folds is more susceptible than other parts of the head; and as closure progresses, the region of high susceptibility moves posteriorly and then disappears. As the optic lobes develop, they become temporarily more susceptible than other parts of the head, and later the otic primordia also appear as regions of high susceptibility. Preceding torsion, the hind-brain region again becomes highly susceptible, a change probably associated with torsion and disappearing later. Susceptibility becomes high in the primordium of the heart, decreasing anteriorly from the posterior end, where the sinus, the dominant region of the heart, finally develops (Hyman, 1927*b*). As the tail bud develops, high susceptibility appears in it, and the limb buds become highly susceptible as they appear. In general, except where lateral organs become localized regions of high susceptibility, the embryo shows decrease in susceptibility from the median region laterally. The remarkable correspondence of regions of high susceptibility to agents as different in their action as potassium cyanide, ammonia, sodium hydroxide, and ultra-violet light with regions of rapid dye reduction and, so far as observations go, with regions of high susceptibility to Janus green is highly significant not only as evidence of the changes in physiological pattern as development proceeds but also as indicating that differential susceptibility shows certain real physiological differentials and their changes. The results suggest that activation is an essential factor, in the early stages of development, not only of the whole embryo but of particular regions and organs.

MAMMALS

There is, at present, no direct evidence bearing on the question of physiological pattern in early mammalian development, but much indirect evidence from the occurrence of teratological forms, many of which are similar to the differential modifications of development under experimental conditions in other vertebrates described in the following chapter. Moreover, there is adequate ground for the belief, very generally held, that there are fundamental similarities in developmental pattern among vertebrates and that the differences in course of development are, in large part, dependent on presence and amount of yolk in fishes, amphibians, reptiles, and birds and its absence, together with uterine development, in mammals.

Functional patterns of various organs of adult mammals indicate a gradient pattern of some kind as an underlying factor. The heart with its dominant region at the sinus end, the high end of a gradient in early

cardiac development of birds (Fig. 56), is an example. The mammalian alimentary tract, particularly the small intestine, is another. There are several regions of relative dominance along the tract, beginning with the pharynx. These may function separately, or temporary subordination of one or more may occur. In the small intestine gradients of irritability, latent period, muscle tone, rate of rhythmic contraction of isolated pieces, susceptibility, and respiration have been demonstrated, all corresponding in direction, but some temporarily reversible in direction by local stimulation at some level below the dominant region at the pylorus. There is a remarkable similarity in functional pattern of the mammalian intestine and the ctenophore plate row (see pp. 106, 327).³⁸ The ureter is another organ in which there is evidently a very similar pattern of function. The central nervous system shows various evidences of a gradient pattern, as might be expected from its close developmental association with the gradient pattern of early stages.³⁹

CONCLUSION

The data of this chapter, though incomplete or fragmentary and often only indicative or suggestive rather than conclusive, constitute, on the whole, a remarkably consistent body of evidence. While complete agreement is lacking on various points, the high degree of parallelism of results obtained on the same material with different methods is sufficiently obvious. There is little room for doubt that the methods are not all concerned with the same aspects of protoplasmic differentials or gradients, but it does seem evident that they are concerned with different aspects of the same pattern. The spatial patterns of early development are not merely patterns of physical condition, of structure, of concentration of substances, of metabolism, but of all of these—in short, they are spatial patterns of living. If this is true, we shall learn all that they are only as we learn all that living protoplasms are. Whatever they are, metabolism appears to be the effective factor in the progress of development. Without it physical structure and chemical substances would not bring about development. Moreover, not only metabolism, but metabolism in a spatial pattern, is essential to orderly axiate development.

Most of the evidence available indicates or suggests that this pattern is primarily quantitative, or at least that differences in rate of living play primarily a more important part in development than differences in

³⁸ See Alvarez, 1928, and his citations of special papers.

³⁹ See, e.g., Herrick, 1924; Coghill, 1929.

kind of living. They appear to be the primary factors in bringing about axial and regional differences in rate of development and in form and proportions.

Eggs of different animal species differ greatly at the beginning of embryonic development: in some there is apparently little or nothing more in the way of developmental pattern than differentials in rate; others give direct or indirect evidence of more or less specific regional differentiation in the cytoplasm. Even in these, however, the cytoplasmic differentiations apparently are, or become, localized in definite relation to a general gradient pattern and are probably to be regarded, like other features of development, as expressions or consequences of that pattern, appearing in the egg rather than in later stages (see chap. xiv). Gradient pattern along any physiological axis is itself a three-dimensional pattern, a gradient system; it may perhaps be regarded as a general background on which details are gradually filled in, or as a frame of reference within which, and in relation to which, developmental events occur. But unless the following pages are wholly in error, it is more than a background or a frame of reference; it is a physiologically active and effective factor in initiating the order and unity of the individual.

CHAPTER V

DIFFERENTIAL MODIFICATION OF DEVELOPMENT: COELENTERATES AND FLATWORMS

IN SO-CALLED "normal" development only certain developmental potentialities are realized; it is possible to alter the course of realization experimentally by altering environmental conditions and so to alter form and proportions of parts, localization of particular differentiations, and even the general patterns of symmetry, asymmetry, and polarity. We call the individuals thus produced "abnormal forms," "monsters," "terata"; but it is scarcely necessary to point out that *normal* development represents the reactions of the protoplasm concerned to a particular environment and *abnormal* development its reactions to other environments.

Differential susceptibility was discussed in chapter iii, but certain points concerning its relation to development are briefly recalled to attention here. Experiment has shown for many animal species that with exposure in early developmental stages to an external inhibiting factor in sufficiently high concentration or dosage to bring about more or less inhibition of all parts, but not rapidly lethal, the inhibition of development is differential—in other words, a gradation in degree of inhibition, decreasing from the most to the least susceptible regions is evident. So far as adequate data are available, the gradient pattern indicated by differential inhibition of development is, in general, essentially the same as that indicated by other methods and is definitely related to the axiate pattern of the organism (see chap. iv).

With a certain lower range of concentration or dosage differential tolerance or acquirement of increased tolerance (a differential conditioning) to many agents appears sooner or later following a primary inhibition. Under these conditions differential modification of form and proportions is opposite in direction to that in differential inhibition. The regions most inhibited primarily show secondarily the greatest tolerance or conditioning. After temporary exposure to the inhibiting agent, provided its action has not produced persistent injury, a differential recovery may occur, with modification of development similar, in general, to that in differential tolerance and conditioning but often more extreme. If the more sus-

ceptible regions are so injured that they cannot recover, the less susceptible may still be able to recover to some extent; it seems desirable to distinguish this, as partial recovery, from the differential recovery of the whole, in which the regions primarily the most susceptible show the greatest recovery. Differential acceleration of development has also been observed in some forms with certain agents. As pointed out in chapters iii and iv, the appearance of the same differentials or gradient patterns with many different agents, both physical and chemical, suggests that differential susceptibility depends primarily on factors of rate rather than of kind of activity, the most active regions being primarily most susceptible to the more extreme effects of external factors and most capable of tolerance or conditioning to, and recovery from, less extreme effects and in some cases most susceptible to accelerating effects. In other words, differential susceptibility appears to be, to a high degree, nonspecific for different agents and apparently depends on quantitative factors of gradient pattern rather than on specific regional differences in the organism, if such are present, or on the nature of the agent and the particular manner in which it acts on living protoplasms.

The data on experimental modifications of early development resulting from exposure of the entire intact organism to altered environmental conditions indicate that most, if not all, of the modifications thus produced depend on nonspecific differential susceptibility and fall under one of the above heads (Child, 1924*a*, 1928*d*). Moreover, it appears that physiological inhibiting factors of various sorts act differentially on development in the same way as external factors. The present chapter and the two following are chiefly concerned with some of the differential modifications of development and their bearing on the problem of pattern.

DIFFERENTIAL MODIFICATION OF HYDROID DEVELOPMENT

Differential inhibition of embryonic development of the hydromedusa *Phialidium gregarium* has been effected by KCN, LiCl, phenyl urethane, dilute neutral red, and CO₂ with essentially similar modification by all agents (Child, 1925*b*). In normal development cells from the basal region of the blastula immigrate singly into the blastocoel to form entoderm (Fig. 57, *A-C*). Under the inhibiting conditions the number of immigrating cells increases, the region of immigration extends farther apically (Fig. 57, *D*), and, instead of forming a single cell layer of entoderm, the immigrating cells may form a solid mass, more or less completely obliterating the blastocoel (Fig. 57, *E-G*). Cells may also be given off

externally as well as internally, usually from the basal region (Fig. 57, *H, I*), occasionally over most of the surface. This emigration occurs more frequently and to a greater extent in LiCl than in other agents used. The cells given off are normal in appearance, with no evidence of cytoly-

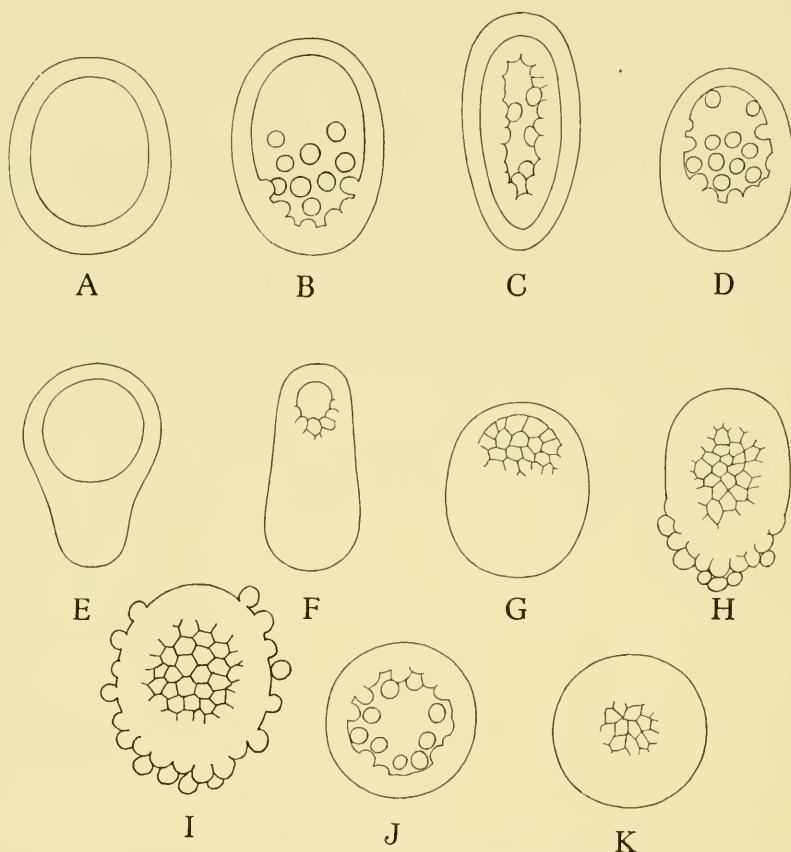


FIG. 57, A-K.—*Phialidium gregarium*. Normal and differentially inhibited development. A-C, normal development of planula; D, E, differential inhibition in early stage with increase in number of immigrating cells and extension apically of region of immigration; F, G, differentially inhibited early planulae, excessive immigration; H, I, immigration and emigration; J, K, obliteration of polarity, equal immigration from all regions resulting in solid spherical mass (from Child, 1925*b*).

sis; the solid planulae remain alive and, as will appear, may develop further. With more extreme inhibition in early blastula stages all evidences of polarity are obliterated, immigration occurs equally from all parts of the wall, giving rise to completely solid spherical larvae without visible structural differences of any kind (Fig. 57, *J, K*). Ciliary co-ordination

or control is evidently lost in these forms; locomotion is limited to an indefinite rolling-about instead of definitely directed with apical end in advance, as in normal planulae. If returned to water, these forms may live 2 weeks or more without further development and without any indication of a developmental pattern. Apparently they are unable to develop unless a new pattern is determined in them (see pp. 425-26). Except for the more frequent emigration of cells in LiCl the same modifications appear as with other agents used. All of them "entodermize"; that is, the higher gradient levels are more or less completely inhibited down to the

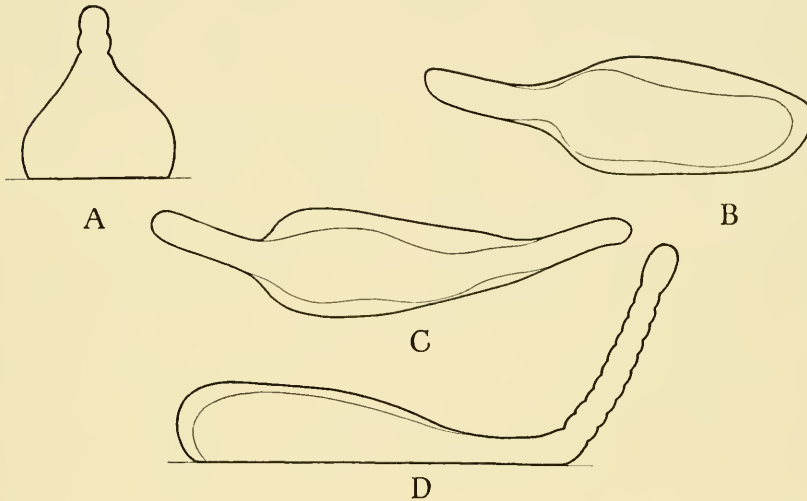


FIG. 58, A-D.—Normal and inhibited development of axes in *Phialidium*. A, normal attachment of planula by end originally apical, and development of hydranth-stem axis from end originally basal; B, C, inhibited planulae with some degree of differential tolerance or differential recovery, giving rise to stolon axes; D, hydranth-stem axis developing from planula stolon after recovery (from Child, 1925b).

level characteristic of the basal region in normal individuals, so that immigration of cells is not limited to the basal region but occurs farther apically or, in the spherical forms, equally from all parts of the wall, and some cells may emigrate instead of immigrating. The entodermization resembles that occurring in echinoderms, and the emigration of cells is probably comparable to echinoderm exogastrulation (see chap. vi).

With lower concentrations of inhibiting agents which do not completely obliterate polarity, retarded elongation of the planula occurs. The normal planula in good condition swims actively and attaches by the apical end (Child, 1925a), and the first hydranth develops from the original basal end (Fig. 58, A). The slightly inhibited planulae do not swim free but

move along the bottom of the container and finally come to rest with one side, instead of the apical end, in contact. After several days indications of differential conditioning, or of differential recovery if they are returned to water, appear; these consist in outgrowth of stolons from the apical end or from both ends (Fig. 58, *B*, *C*), representing either the primary gradient of the planula or both gradients (pp. 96-97). They may continue to grow as stolons at the expense of other parts of the planula body or, if returned to water, may recover sufficiently to give rise to a hydranth-stem axis (Fig. 58, *D*). Failure of the planulae to attach by the apical end and development of stolons instead of hydranth-stem axes from one or both ends occur with very slight degrees of inhibition—for example, in slightly crowded cultures or with insufficient aeration, as well as with low concentrations of various chemical agents.

The normal development of the gymnoblast hydroid *Corymorpha* after attachment of the planula is shown in Figure 59, *A-C*. As indicated in Figure 30 (p. 98), the high end of the primary gradient is apical, and a secondary gradient appears basally in the course of development. The egg of *Corymorpha* is so opaque that effects of differential inhibition on early development are obscured; but polarity can apparently be obliterated, as in *Phialidium*, by cyanide, lithium, alcohol, ether, and probably by methylene blue and neutral red, the embryo remaining spherical within the membrane, though alive. With less extreme inhibition, retarding development but not preventing hatching and not obliterating polarity, the planulae become much longer than normal because the less susceptible basal and middle regions are less inhibited than the apical end (Fig. 59, *D*, *E*). Further development of these forms in the inhibiting agent is indicated in Figure 59, *F-H*. If tentacles appear at all, they do not develop beyond early stages; distal tentacles are more inhibited than proximal and often do not form (Fig. 59, *F*); often development does not go beyond the stages of Figure 59, *D* and *E*. In all cases the hydranth region is small and the stem large and elongated. Evidently the less susceptible stem has been able to grow to a greater extent, as compared with the hydranth, than in normal development. If the forms of Figure 59, *F-H*, are returned to water, hydranth development proceeds, the hydranth growing at the expense of the stem, so that the usual proportions are approached, that is, differential recovery occurs. With slightly greater inhibition development rarely goes beyond the stages of Figure 59, *I* and *J*, except after return to water.

Differential regression of development may also be brought about by

inhibiting agents. Stages like Figure 59, *B* and *C*, may undergo regression to forms essentially like *F*, *G*, and *H* and develop again if returned to

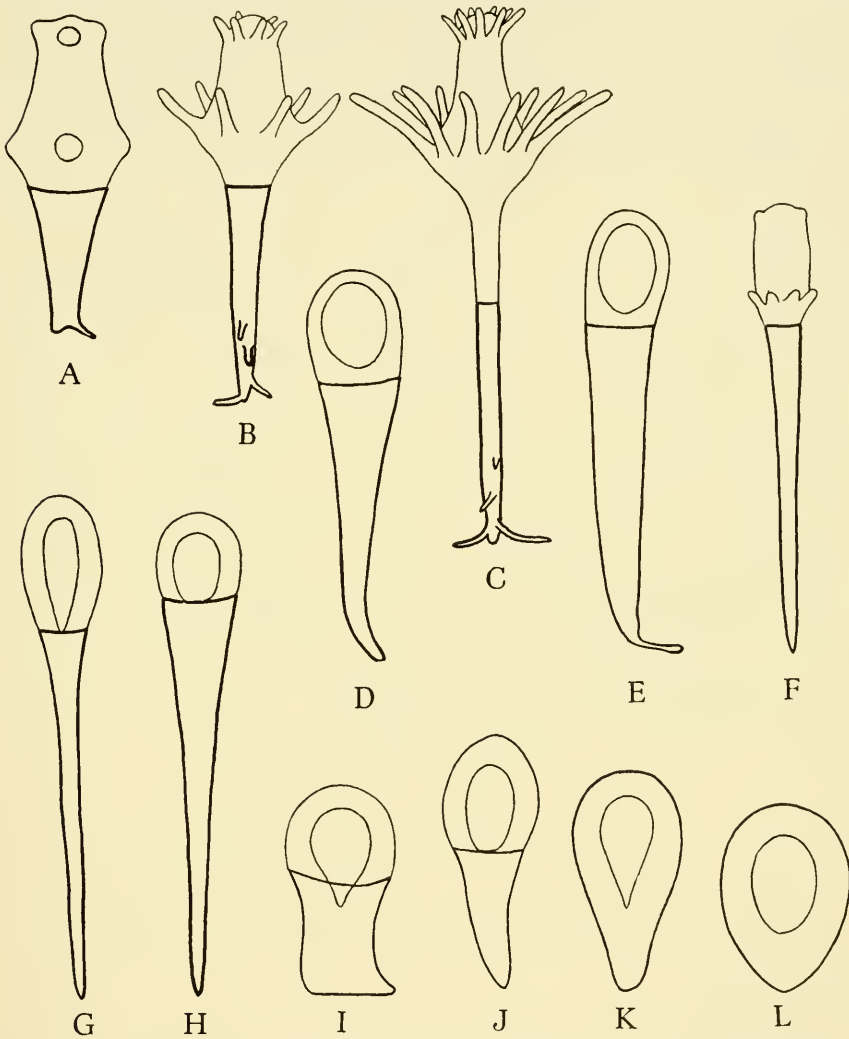


FIG. 59, *A-L*.—Normal and differentially inhibited development of hydroid of *Corymorpha palma* from planula. *A-C*, stages of normal development; *D*, *E*, differentially inhibited development in planula stage; *F*, *G*, *H*, later stages of differentially inhibited development; *I*, *J*, more extreme inhibition; *K*, *L*, regression from stage of *B* to planula-like stage.

water. Regression of stages like Figure 59, *B*, to forms resembling early planulae completely inclosed in perisarc is possible with inhibiting agents,

but renewed development of these forms following return to water has not been observed. These regressions take place without visible disintegration, but the tentacle cells are apparently resorbed and may serve as nutrition. No evidence of specificity of the various modifications for any agent used has been found, and comparison with the lethal susceptibilities (pp. 101-4) and with results of differential dye reduction shows that the same gradient pattern is indicated by the different methods.¹

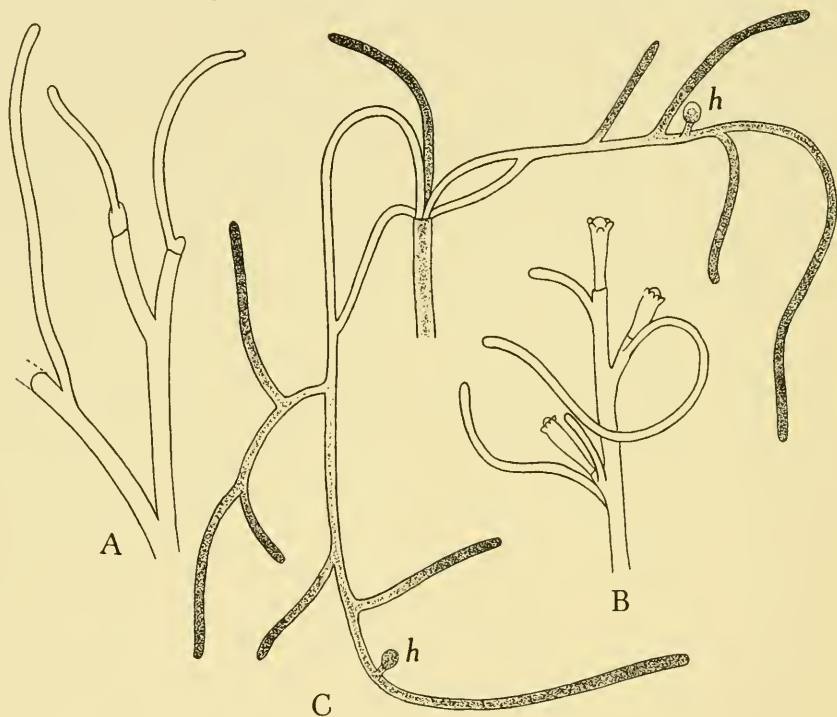


FIG. 60, A-C.—*Bougainvillia mertensii*. Stolon development from hydranth-stem axes under inhibiting conditions. A, 48 hr. in ethyl urethane m/200; B, KCN m/50,000; C, KCN m/50,000 for 6 days, then KCN m/100,000 with further decrease in concentration for 8 days (from Child, 1923a).

The transformation of hydranth-stem axes into stolon axes in hydroids is of interest as a differential inhibition in later stages of these forms (Child, 1923a). From the study of many hydroid species it appears that hydranths are much more susceptible than stolons and that the motile hydranth is more susceptible than the nonmotile bud. In some hydroid species stolons are not infrequently found developing from apical ends of

¹ The data on *Corymorpha* have not been previously published.

branches, particularly in the more basal parts of the system, under natural conditions. Experiment throws light on the character of these transformations. Certain highly susceptible hydroids, e.g., *Bougainvillia* and *Plumularia*, when kept in standing water in the laboratory, lose their hydranths in a few days, sometimes in a few hours—the fully developed hydranths usually by disintegration, the buds apparently by regression and resorption. A few days later stolons develop in place of hydranths, even from the apical ends of axes, the whole system often showing nothing but

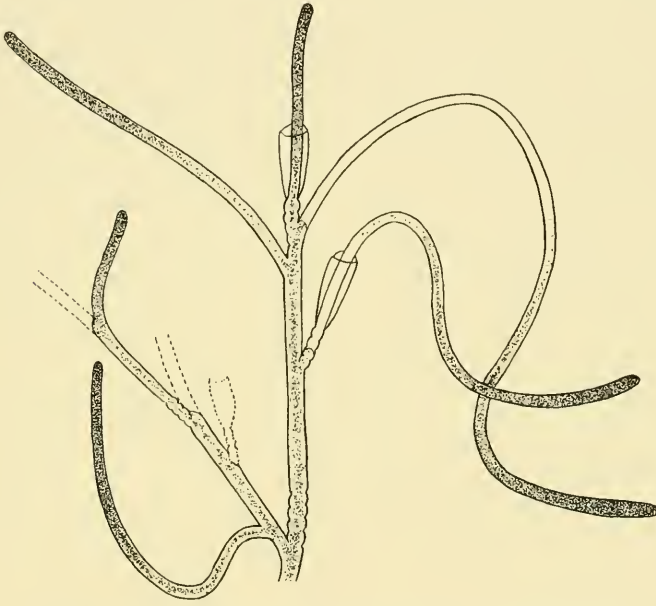


FIG. 61.—*Gonothyraca clarkii*. Stolon development from hydranth-stem axes in standing water (from Child, 1923a).

stolons. Still later, under the same conditions, appearance of hydranth buds suggests some degree of conditioning. With low concentrations of inhibiting agents these transformations may be even more rapid. In ethyl urethane m/200 *Bougainvillia* may undergo complete transformation in 48 hours (Fig. 60, A). In KCN m/50,000 the stolons are often subapical, and after a week or more the appearance of hydranth buds indicates some degree of conditioning, but the buds do not develop beyond an early stage (Fig. 60, B). A stolon system developing in cyanide from a single apical end is shown in Figure 60, C. Transformation in *Gonothyraca* is slower but may take place in standing water (Fig. 61); in *Obelia*

and several other species similar transformations occur. Ethyl urethane, cyanide, MgSO_2 , LiCl , neutral red, and CO_2 all give essentially similar results. Pieces of *Plumularia setacea* (California) in standing water lose the original hydranths and develop numerous stolons from lateral branches and from both cut ends of the chief axis, and no, or very few, hydranths. In flowing water hydranths remain and hydranth-stem axes develop from one or both cut ends of the chief axis, no stolons or very few appearing. *Sertularella miurensis* (Japan) in standing water loses hydranths and develops lateral and terminal stolons. In hypotonic standing sea water, 75 and 50 per cent, and in flowing normal sea water hydranths remain alive, and few or no stolons develop until inanition is far advanced.

These transformations of hydranth-stem axes into stolon axes obviously result from differential inhibition. The most susceptible parts, the hydranths, die or are resorbed, and the inhibited axes develop stolons. With differential conditioning or recovery the stolons may give rise to hydranths. Each stolon represents a growth gradient, the tip growing and remaining in good condition, at the expense of other parts, in the absence of food or after separation from the stock. As the stolon increases in length, separation from the parent stock occurs because the cells at the low end of the stolon gradient serve as food for cells nearer the tip. The tip continues to grow at the expense of more proximal levels until reduction of the coenosarc to very small size. In Figures 60 (C) and 61 the graded shading of the stolons indicates the gradation from the tips, where cells are in good condition and fill the perisarc completely, to levels where only a slender coenosarc strand of atrophying cells persists. Unshaded parts of these figures indicate empty perisarc. The stolon axis is a growth gradient, the hydranth-stem axis is a differentiation gradient; the stolon axis develops under somewhat depressing or inhibiting conditions, the hydranth-stem axis under conditions more nearly optimal. The inhibiting factor may be external, as it undoubtedly is in the transformations of apical regions into stolons in standing water and in slightly toxic solutions; or it may be physiological, as in determination of stolon development at proximal levels of an axis by dominance of the hydranth-region (see pp. 314-15). In any case, stolon development evidently indicates a relatively low level of certain metabolic reactions. Oxygen is probably often insufficient to support hydranth metabolism at the more basal levels of hydroid systems as their own stems and branches become more numerous or in consequence of overgrowth by other forms. Many facts indicate that the oxygen content of sea water under natural conditions is often not far

above the critical level for hydranth development in various hydroid species.² When it falls below that level, stolons develop in some forms; in others there is no development, at least under laboratory conditions, but insufficient food or other incidental conditions may be responsible for its absence. Doubtless stolon metabolism is different from hydranth metabolism, but apparently a low level of the kind of metabolism which brings about hydranth development makes stolon development possible.

DIFFERENTIAL MODIFICATION OF PLANARIAN RECONSTITUTION

Isolated planarian pieces provide interesting and valuable material for the study of differential developmental modification in its relation to developmental pattern. In these pieces it is possible to determine the differential effects not only of external factors but also of certain physiological conditions. Pieces of different lengths from different body-levels, from individuals of different physiological age, nutritive condition, previous conditioning, etc., provide somewhat different starting-points for physiological analysis. Moreover, the differential modifications are not limited to the longitudinal axis.

LONGITUDINAL MODIFICATIONS

Under natural conditions regeneration of new tissue is more rapid at the anterior than at the posterior end of the piece in *Dugesia* (see Figs. 17, 18), and the amount of new tissue formed in head-development is apparently greater than in development of the posterior end. When pieces are subjected immediately after section to concentrations of agents which inhibit reconstitution but are not lethal, development of new tissue is inhibited at both ends, apparently with little, if any, difference. At this time, when activation of the cells near the cut ends is occurring, there is apparently little difference in condition at the two ends. However, if the concentrations of inhibiting agents do not completely inhibit regeneration, the anterior new tissue gradually begins to grow and slowly develops into a head, which may be normal or more or less differentially inhibited, while posterior regeneration is almost or quite inhibited. Under these conditions pieces which in normal environment give rise to complete individuals develop into tailless forms, often without pharynx (Fig. 62, *A, B*). In these more new tissue appears anteriorly than posteriorly, but less than in normal head development; and the head is formed in greater or less part from the old tissue of the piece (Fig. 62, *B-D*) instead of entirely

² H. B. Torrey, 1912; Child and Watanabe, 1935*b*; Barth, 1937*b*; J. A. Miller, 1937.

from the regenerated tissue, as normally. In these cases the anterior regenerating tissue of the piece apparently undergoes conditioning more rapidly, or to a greater degree, than the posterior end. These secondary modifications have been obtained with KCN, ethyl alcohol, chloretone and other anesthetics, CO₂, hydrogen ion, and low temperature. With cyanide they develop very slowly, usually becoming evident only after several weeks' exposure; in alcohol they begin to appear in a few days; with other agents used, they develop at different rates between these extremes.

The history of these pieces suggests that immediately after section there is little difference in condition at the two cut ends, but later there is evidently a difference. If the pieces remain in water 48 hours after section and are then subjected to the inhibiting agent, anterior regenera-

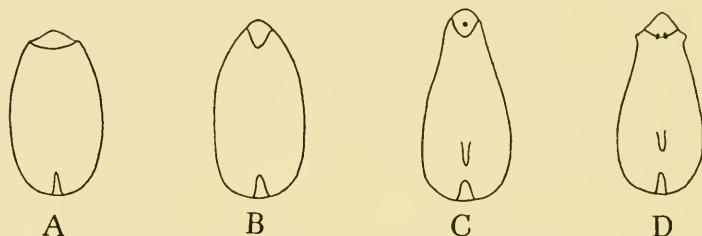


FIG. 62, A-D.—Anteroposterior differential tolerance and differential conditioning to inhibiting agents in pieces of *Dugesia dorotocephala*. A and B, early, C and D, later, stages of differential tolerance or conditioning, showing greater inhibition of posterior reconstitution.

tion is more inhibited than posterior; that is, a direct differential inhibition occurs. This is more clearly evident with agents such as cyanide, in which the secondary modification occurs very slowly. Measurements of length and width of anterior and posterior new tissue make it possible to compare areas of anterior and posterior regenerating tissue in controls and experimental pieces; since thickness of the two regions is much the same, the measurements serve as a rough ratio of amounts of anterior and posterior regenerated tissue. The following data serve as examples:

	$\frac{\text{Anterior regeneration}}{\text{Posterior regeneration}}$
Controls, 8 days in water.	1.43*
Experiment { 2 days in water, 6 days in KCN m/100,000. . .	1.11
{ Same lot after 14 days in KCN m/100,000. . .	1.66

* The figures are the sums of products of lengths and widths of anterior, divided by the sums of products of lengths and widths of posterior regenerated tissue, as determined in each piece of the experimental lot.

In the experimental lot the ratio of anterior to posterior regeneration after 6 days in cyanide is far lower than in the control, that is, inhibition

is greater anteriorly than posteriorly; after 14 days in cyanide the anterior-posterior ratio in the same pieces is considerably higher than in the control, the conditioning of the anterior region to cyanide being apparently greater than that of the posterior. These differences are directly visible and often become greater in later stages, but these have not been measured. With some agents—for example, ethyl alcohol—the secondary modification occurs so rapidly that the differential inhibition preceding it is slight or scarcely appreciable. In general, the greater susceptibility of the regenerating head region than of the posterior regenerate to lethal concentrations or dosages and its more rapid or greater conditioning in a lower range of concentrations are conspicuous characteristics of planarian pieces undergoing reconstitution. That they are expressions of a longitudinal pattern appears beyond question.

HEAD FORMS AND HEAD FREQUENCIES IN PLANARIAN RECONSTITUTION

In the reconstitution of pieces of a number of planarian species graded series of head forms develop, ranging from the normal fully developed head to the completely acephalic condition. These head forms are evidently expressions under different conditions of a differential or gradient pattern in the parent body and persisting in the regenerating head with both anteroposterior and mediolateral components. On the one hand, the head forms constitute a continuous series of differential inhibitions of head development; on the other, a series of secondary modifications representing differential conditioning and recovery. Chief attention is given to the inhibition series because of its relation to physiological, as well as external, factors, because the degree of differential inhibition and consequently the head form can be experimentally altered and controlled in many ways, and because this series has thus far provided a basis for a wide range of experimental analysis. The secondary modifications, though physiologically equally significant, appear under rather narrowly limited experimental conditions; they will be considered in a later section.

Head forms.—The head forms of the inhibition series have been classed in five groups for convenience, but it must be remembered that the limits of these groups are arbitrary and that the series is actually continuous. The groups are as follows:

The normal head: The head form which is typical of the species in natural environment is triangular in general outline, with two separate, bilaterally localized “eyes” (photoreceptors) and two cephalic lobes (chem-

oreceptors) on lateral margins of the head at a level slightly posterior to that of the eyes. The head form is shown in Figure 63, *A*, a transverse section of head and ganglia at eye-level in Figure 64, *A*.

The teratophthalmic head: In animals developing from pieces of equal length from the same body-level of the parent teratophthalmic heads are usually slightly smaller than normal heads and often less sharply pointed

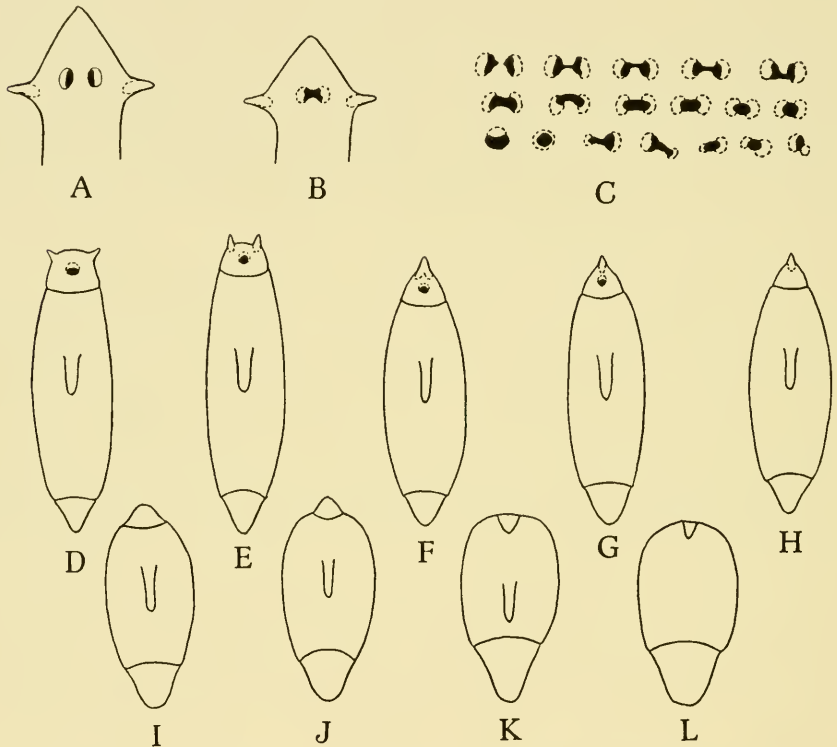


FIG. 63, *A-L*.—The inhibition series of head forms of *Dugesia dorotocephala*. *A*, normal head; *B*, teratophthalmic head; *C*, eyes of teratophthalmic heads; *D-G*, teratomorphic heads; *H-J*, anophthalmic heads; *K*, *L*, acephalic forms.

anteriorly and shorter, at least in earlier stages. The eyes are localized nearer the median line than normally, and the pigment cups are more or less connected; all degrees of approximation, even to complete cyclopia, occur in these forms (Fig. 63, *B*, *C*). The two eyes are sometimes unequal and localized at somewhat different levels, but these irregularities apparently result from incidental differences on the two sides of the piece; for example, the presence of an intestinal branch at the level of section

on one side may determine slightly slower growth on that side because parenchyma cells are less numerous. Figure 64, *B*, shows that the median ganglionic region is apparently absent and that the more lateral parts of the ganglia are approximated to the median line and partially fused.

The teratomorphic head: This head is rounded anteriorly with median eye, apparently single (cyclopia),³ and with cephalic lobes localized more or less anteriorly instead of laterally and showing all degrees of approximation to the median line, with a single median lobe as the extreme (Fig. 63, *D-G*). The ganglionic modifications correspond in degree to those of other parts of the head (Fig. 64, *C, D*). In the extreme teratomorphic

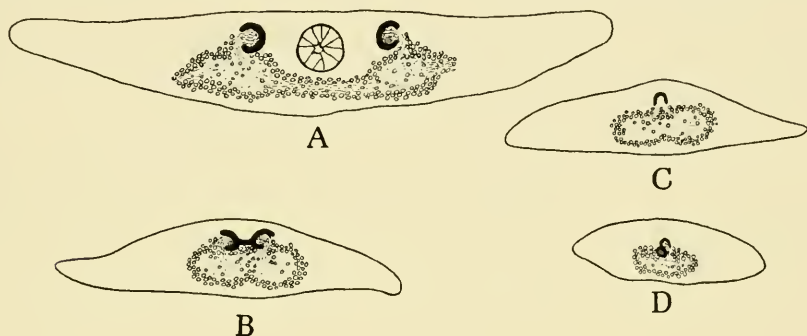


FIG. 64, *A-D*.—Transverse sections of normal and differentially inhibited heads. *A*, normal head of large animal; *B*, teratophthalmic; *C, D*, teratomorphic, reconstituted heads (from Child and McKie, 1911).

forms (Fig. 63, *G*) the region corresponding to the normal head is only a narrow median band, the median parts of the normal head not being represented at all; the originally lateral regions of the normal head are now anterior and near, or in the median plane.

The anophthalmic form: In these forms the anterior regenerate may resemble the extreme teratomorphic head with a single median cephalic lobe but is without an eye (Fig. 63, *H*), or the head may be represented only by an outgrowth of new tissue without distinguishable external differentiation (Fig. 63, *I, J*). The ganglionic mass shows various degrees of more extreme reduction than in the teratomorphic head, and in some cases only traces of ganglia appear at best; and it might even be ques-

³ In sections two pigment cups forming a rounded mass and two nerves, side by side or practically united, or two pigment cups, one ventral to the other, are sometimes distinguishable; but usually there is only one (see Fig. 64, *C, D*).



tioned whether the outgrowth should properly be called a head. However, even forms like Figure 63, *I* and *J*, behave much more nearly like normal animals than do the completely acephalic forms.

Acephalic forms: In these the anterior cut surface becomes strongly contracted and a small amount of new tissue fills in the concavity and differentiates into a continuation of the lateral margins which bound the contracted cut (Fig. 63, *K*, *L*). There is no trace of cephalic ganglia or of eyes, and locomotion usually continues only a short time when induced by strong stimulation. These forms can be distinguished, even from

anophthalmic forms, by their locomotor behavior. A pharynx and mouth develop in acephalic forms from prepharyngeal levels (Fig. 63, *K*) but, so far as observed, never in those from postpharyngeal levels (Fig. 63, *L*).

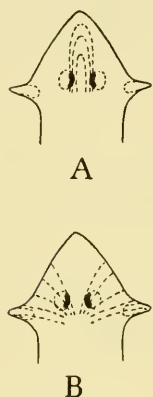


FIG. 65, *A*, *B*.—Corresponding broken lines on the two sides indicate approximately the regions most reduced or absent in teratophthalmic (*A*), teratomorphic and anophthalmic (*B*), forms.

In Figure 65, *A*, the parts of the head which are absent in different degrees of teratophthalmia are approximately those lying between the corresponding broken lines on the two sides of the median plane. Figure 65, *B*, indicates in the same way the regions absent in teratomorphic and anophthalmic forms. The mediolateral differential susceptibility determines this differential inhibition, progressing from the median region laterally. Particular attention is called to the fact that the inhibition is not specific for particular organs of the head but is regional, involving those parts of cephalic organs which happen to lie in the region concerned. Actually, of course, the inhibition occurs before the organs have differenti-

ated; but when differentiation occurs, the relation of particular organs to certain regions is clear. When the median head region is inhibited, the median region of the cephalic nervous system does not develop elsewhere but is absent; with inhibition extending farther laterally the ganglionic defect extends farther laterally, the eyes are approximated, or cyclopia or anophthalmia results, and the lateral cephalic lobes show all degrees of approximation to the median line, finally a single median lobe; in anophthalmic forms there are only the rudiments of head development. In short, this differential inhibition of the planarian head shows no relation to particular organs or their parts but simply eliminates regions progressively from the median plane laterally. Other differential inhibitions to be described later show similar characteristics.

Head frequencies.—The frequency of occurrence of the various degrees of differential inhibition of the anterior end in a particular lot of pieces of the same length from the same body-level of animals of a certain length and as nearly as possible in similar physiological condition has been called, for convenience, “head frequency.” In such lots head frequency shows a high degree of constancy, but it is a rather sensitive indicator of differences in physiological condition and in external environment. With animals of a certain length from a particular locality, which probably signifies similar nutritive condition, possibly in some cases conditioning to some external factor, and with similar laboratory environment, head-frequency values differ in definite directions with length of piece, level of body, and conditions under which reconstitution occurs. In pieces from animals of different length the values also differ in definite directions, with fraction of body length represented. Moreover, they differ in characteristic ways with differences in nutritive condition of the animals and with differences in temperature and other factors of the environment of the stocks preceding section. Animals from different localities or from the same locality at different seasons of the year may give characteristically different frequencies. Acclimating or conditioning animals to certain environmental factors preceding section alters the frequencies. And finally, so far as comparison is possible, different species of the American genus *Dugesia* (= *Euplanaria*), and at least some species of the genus *Planaria*, show characteristic species differences in head frequency.⁴

HEAD FREQUENCY IN RELATION TO CERTAIN PHYSIOLOGICAL FACTORS

The relation between head frequency, length of piece, and body-level is shown graphically for *Dugesia dorotocephala* in Figure 66, in which the head-frequency indices for pieces $1/3$, $1/4$, $1/6$, and $1/8$ of the post-cephalic body length are plotted as ordinates against body-levels as abscissae. As the graph shows, head frequency decreases from the anterior level to the region of the fission zone, that is, in the anterior zooid, scarcely at all in $1/3$ pieces, more in $1/4$, still more in $1/6$, and most in $1/8$ pieces. At the most anterior level the frequency is the same in pieces of all lengths, but decreases more steeply as length of piece decreases. From the region of the fission zone it increases posteriorly. With still shorter pieces the decrease and increase are still steeper, but with sufficiently short pieces the most anterior and posterior levels show a decrease in frequency, as compared with longer pieces (Child and Watanabe, 1935a). In other

⁴ For the literature chiefly concerned with planarian head frequency, the method used for obtaining a “head-frequency index,” and the application of statistical methods to evaluation of head-frequency data, see Appendix VII (p. 745).

words, the head frequency at any level decreases with decrease in length of piece below a certain limit, but this limit differs widely at different body-levels. The length of piece at which differential inhibition of head development begins to appear increases posteriorly in the anterior zoid

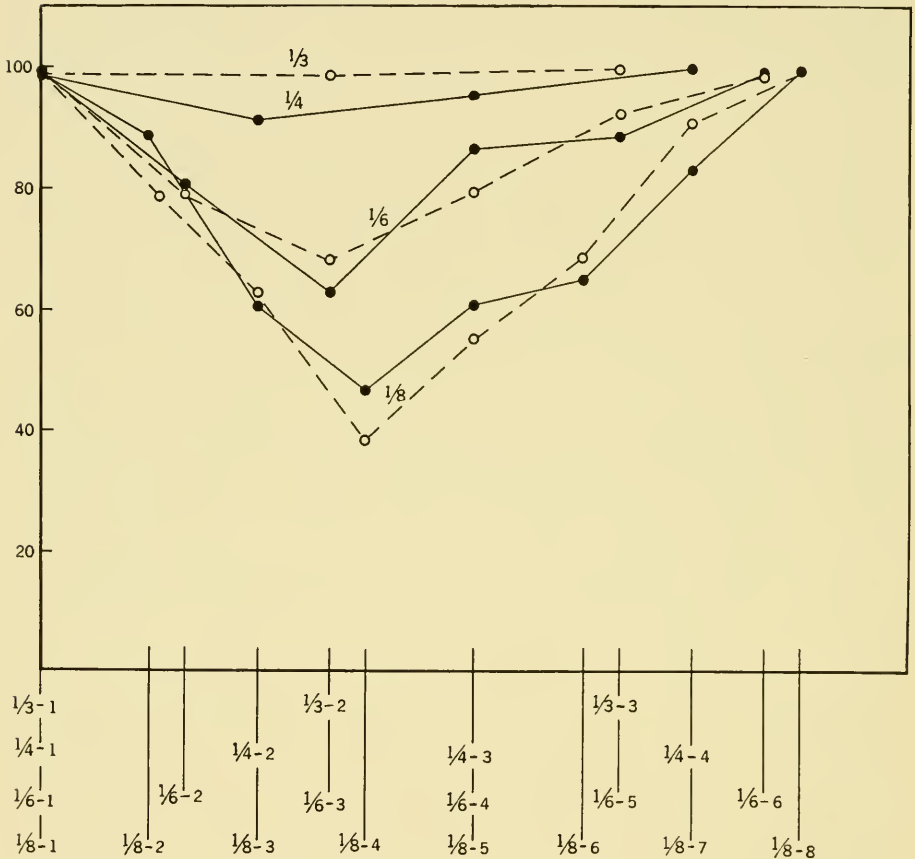


FIG. 66.—Head frequencies of $1/3$, $1/4$, $1/6$, and $1/8$ pieces of postcephalic regions of *Dugesia dorotocephala*. Head-frequency indices plotted as ordinates against body-levels as abscissae; unbroken and broken lines represent frequencies obtained by different groups of students in different years.

and decreases again in the posterior-zoid region. In these lots of pieces only head forms of the inhibition series appear, except for occasional secondary modifications indicating differential tolerance, conditioning to, or recovery from, the inhibiting factor, and slight differences in size, position, and time of appearance of the two eyes, evidently due to incidental differences on the two sides of the cut end.

The physiological factor determining the graded series of head forms in pieces below certain lengths is an effect resulting from section at the posterior end of the piece and the following stimulation of the nervous system and activation of cells there; it is apparently, at least in large part, nervous in character, for section of the nerve cords posterior to the level of head development inhibits the head to almost the same degree as section of the whole body. It is also very clearly shown that the inhibition of the head results from posterior section by delaying either posterior or anterior section for different periods after the other section has been made. Delay of posterior section even for 1 or 2 hours increases head frequency in short pieces; but with delay of anterior section head development is inhibited over a much longer period, complete normal head development being attained only with 3 or 4 days delay.⁵

Head frequencies are usually lower in pieces from animals kept without food for several weeks before section than in similar pieces from well-fed animals. Probably in the starved animals the cells from which the head develops are less intensely activated and therefore more inhibited by the stimulation resulting from posterior section. In pieces representing equal fractions of body length head frequency is less in those from small, young animals, doubtless because the pieces are shorter and the inhibiting factor from the posterior section therefore more effective than in the longer pieces from large individuals (Child, 1911f). In general, animals in good condition give a steeper head-frequency gradient than those in poor condition; this gradient is almost invariably steeper in newly collected animals than after long periods in the laboratory (Rulon, 1937). These data suggest that head frequency may be altered in two different ways: by altering the condition of the cells concerned in head development directly, and by altering the intensity or effectiveness of the inhibiting factor resulting from posterior section and so indirectly altering the condition of the head-forming cells. The data presented in the following section indicate that both these possibilities are realized with external factors.

HEAD FREQUENCY IN RELATION TO EXTERNAL FACTORS

Head frequency may be increased or decreased by environmental factors. With low concentrations of KCN it may be decreased or increased, according to length of piece, body-level of origin, period of exposure, and concentration of cyanide. With the same concentration of cyanide and

⁵ For further discussion of the experiments on section of nerve cords and on delay of anterior and posterior section see pp. 406-11; also Child and Watanabe, 1935a, and Watanabe, 1935b.

the same exposure period it can be decreased in anterior, and increased in posterior, pieces of the same length from the anterior zooid and again decreased in similar pieces from the posterior-zooid region. In general, in pieces with high head frequency in water these concentrations of cyanide decrease it; in pieces with low frequency they increase it (Child, 1916*b*). A few data are presented by way of illustration. Table 6 gives head frequencies in pieces of different lengths and from different body-levels for controls and different exposure periods with several concentrations of cyanide. In the pieces *x* of Table 6 and Figure 67, which are so long that

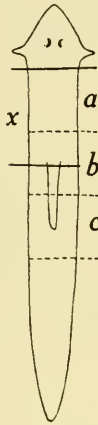


FIG. 67.—Outline indicating lengths and levels of pieces *x*, *a*, and *c* of Tables 6 and 7

the physiological inhibiting factor does not greatly affect head frequency, cyanide decreases it in all concentrations used, and the decrease is greater with continuous exposure to a lower concentration (Table 6, I*x*) than after temporary exposure to a higher (Table 6, II*x*). In shorter pieces, approximately thirds of the anterior zooid (Fig. 67, *a*, *b*, *c*), *a*-pieces show a decrease in head frequency with all concentrations and exposure periods that are at all effective. Three examples are given in Table 6, Ia, II*a*, and III*a*. In the *c*-pieces, on the other hand, continuous exposure to low concentrations of cyanide increases head frequency from the more inhibited forms to teratophthalmic heads (Table 6, I*c*) and with shorter exposures may increase frequency of normal heads (Child, 1916*b*). With higher concentration and continuous exposure increase becomes

less, is not altered, or decrease occurs. In II*c* of Table 6 there is an increase from anophthalmic and acephalic forms to teratophthalmic heads, but there is also a decrease from normal to teratophthalmic; consequently, the index shows no significant change. Shorter exposures (48–72 hours) to the same concentration as II*c* may, however, produce marked increase. But even brief exposures to still higher concentrations decrease frequency in *c*-pieces, and deaths begin to occur (Table 6, III*c*). The *b*-pieces (Fig. 67) which are not included in Table 6 give results intermediate between *a* and *c*. Often they show no significant change in frequency under conditions in which decrease occurs in *a* and increase in *c*.

Within certain limits high temperature increases, low temperature decreases, head frequency; pieces from animals conditioned to a certain temperature give different frequencies from those of animals conditioned

to a different temperature when both lots reconstitute at the same temperature (Behre, 1918). Some effects on head frequency of temperature

TABLE 6

ALTERATION OF HEAD FREQUENCY BY KCN (*Dugesia dorotocephala*)

Fifty pieces in each lot; lots *a* and *c* with same Roman numeral are from the same animals; frequencies in percentages.

Pieces	Concentration and Exposure	Normal	Teratophthalmic	Teratomorphic	Anophthalmic	Acephalic	Dead	Index
I x	control	96	4					99.2
	m/200,000, continuous	32	68					86.4
II x	control	92	8					98.4
	m/100,000, 96 hours	70	30					94
I a	control	84	16					96.8
	m/200,000, continuous	4	80	6	6	2		74.4
II a	control	86	14					97.2
	m/100,000, continuous		76	10	10	4		71.6
III a	control	74	26					94
	m/50,000, 24 hours	52	48					88.4
I c	control	10	28	2	18	42		49.2
	m/200,000, continuous	8	78		8	6		74.8
II c	control	46	26	6	12	10		77.2
	m/100,000, continuous	24	74	2				73.2
III c	control	12	50	8	16	14		66
	m/50,000, 24 hours	8	44	6	20	18	4	58.4

and temperature-conditioning are shown in Table 7. Low temperature before and after section determines a much lower frequency in *a*-pieces than medium temperature before and after section (*Ia*₁, *IIa*₁), but only slightly lower in *c*-pieces (*Ic*₁, *IIc*₁). Pieces from animals conditioned to

low temperature but reconstituting at medium temperature (Ia_2 , Ic_2) show a much higher frequency than those conditioned to, and reconstituting at, low temperature (Ia_1 , Ic_1) and also higher than those conditioned to, and reconstituting at, medium temperature (IIa_1 , IIc_1). Conditioning to medium, with reconstitution at low temperature, results in great decrease in a -pieces with some deaths and decrease with many deaths in

TABLE 7
HEAD FREQUENCY AND TEMPERATURE

Pieces a and c (Fig. 67) of each series from the same animals (50 pieces in each lot); frequencies in percentages. "Low" temperature is 8°-10° C.; "medium," 18°-20° C.; "high," 27°-30° C.

Series and Lot	Temperature before and after Section	Normal	Teratophthalmic	Teratomorphic	Anophthalmic	Acephalic	Dead	Index	
I	a 1	low-low	24	76				84.8	
	a 2	low-medium	86	14				97.2	
	c 1	low-low		14	2	30	50	4	34.4
	c 2	low-medium	10	62	14	8	4	2	72
II	a 1	medium-medium	78	22				95.6	
	a 2	medium-low	44	50			6	84	
	c 1	medium-medium		20	6	24	50		39.2
	c 2	medium-low		2	6	28	40	24	24.4 (32.1)*
III	a 1	medium-medium	76	24				95.2	
	a 2	medium-high	92	8				98.4	
	c 1	medium-medium	2	22	2	28	46		42.2
	c 2	medium-high	28	60	2	6	2	2	80

* Second index for living pieces only.

c -pieces (Ser. II). Series III shows the great increase resulting from reconstitution at high temperature of pieces conditioned to medium temperature. Comparison of IIa_1 with $IIIa_1$ and of IIc_1 with $IIIc_1$ also shows how slight the variation is in similar pieces from similar lots under the same external conditions.

A number of anesthetics have been found to act essentially like cyanide, the same concentration and exposure period decreasing or increasing

head frequency according to level of origin of the piece (Buchanan, 1922). Long periods of exposure to low concentrations of caffeine also decrease head frequency in anterior, and increase it in posterior, pieces, an effect like that of cyanide; but brief exposures to high concentrations may increase the frequency in anterior, and decrease it in posterior, pieces (Hinrichs, 1924a).⁶ Induced increase of motor activity increases head frequency, whether by providing for more adequate respiratory exchange by change in position or by otherwise stimulating the head-forming cells is uncertain (Child, 1911f).

Carbon dioxide, hydrogen ion, and certain organic acids are highly effective in increasing head frequency in posterior pieces of the anterior zooid but have very little effect in decreasing it in anterior pieces (Rulon, 1936a, 1937). The effect of CO₂ is shown in Figure 68. Hydrogen ion in the concentration found effective increases the frequency in the more posterior pieces, where it is low; the increase is much greater in pieces from animals previously conditioned to CO₂ and calcium antagonizes the effect of hydrogen ion (Fig. 69).

The action of strychnine (sulphate) is very similar to that of anesthetics and CO₂. It increases the frequency greatly in posterior pieces but alters it little or not at all in anterior pieces (Figs. 70, 71); it apparently retards development slightly, but the pieces are highly sensitive to stimulation.

⁶ In order to show increase in frequency in anterior pieces, it is desirable to use pieces which give relatively low frequencies under standard conditions—for example, pieces from small young animals, from starved animals, or pieces from large animals, but short enough to show a low frequency.



FIG. 68.—Increase in head frequencies of 1/8 pieces from anterior zooids of 14–16-mm. animals by CO₂ (M) over control (L); indices as ordinates against body-levels as abscissae (from Rulon, 1936a).

Evidently it acts chiefly on the physiological, supposedly nervous, factor which inhibits head development; but, instead of making this factor more effective, it decreases its effectiveness in some way, perhaps by general stimulation of the nervous system with resulting functional disorganiza-

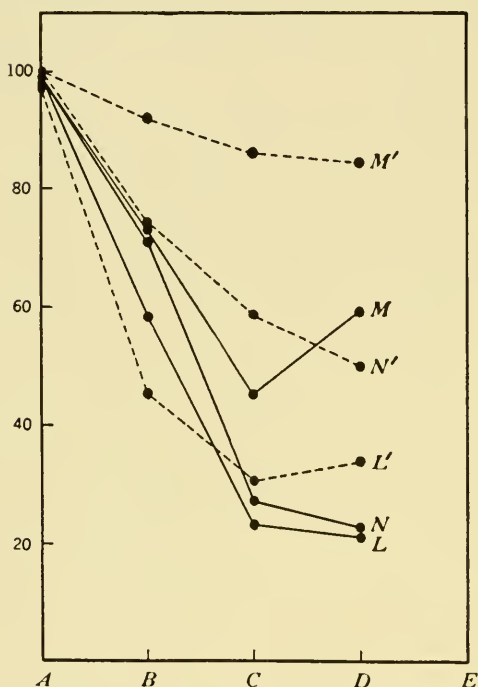


FIG. 69.—Head frequencies of 1/8 pieces from anterior zooids of animals conditioned to CO₂ and non-conditioned animals under different experimental conditions. L, nonconditioned, reconstituted in water; L', conditioned, reconstituted in water; M, nonconditioned; M', conditioned, reconstituted in carbonate-free water, pH 4.15; N, nonconditioned; N', conditioned, reconstituted in carbonate-free water, pH 4.5, plus 0.5 gm. of CaCl₂ per liter. Indices as ordinates against body-levels as abscissae (from Rulon, 1936a).

effective and the gradient is less steep.⁷ Certain other triclad species without posterior zooids show a decrease in frequency of head development from anterior to posterior levels over the whole body length. In some

(F. S. Miller, 1937). With certain concentrations and exposure periods strychnine is so highly effective that pieces from the more posterior levels of the anterior zooid, largely acephalic in the controls, develop a high percentage of normal heads.

In pieces so long that there is no physiological inhibition of head development in controls, cyanide, various anesthetics, caffeine in certain concentrations, CO₂, hydrogen ion, and low temperature all decrease head frequency more or less; that is, inhibited head forms appear in significant percentages, instead of high normal frequency.

The head-frequency gradient of *Dugesia agilis* and *D. tigrina*, both of which have a posterior-zooid region, is essentially like that of *D. dorotocephala*, except that in *D. tigrina* the physiological inhibiting factor is apparently less

⁷ Head frequencies under natural conditions have been determined in the forms earlier known as *Planaria lata* (Sivičkis, 1923), *Euplanaria maculata* (Watanabe, 1935b), and *E. novangliae* (Child), all of which are now regarded by Hyman as *D. tigrina* (see p. 41, footnote 7). A Japanese planarian from one locality was found to give differentially inhibited heads only in

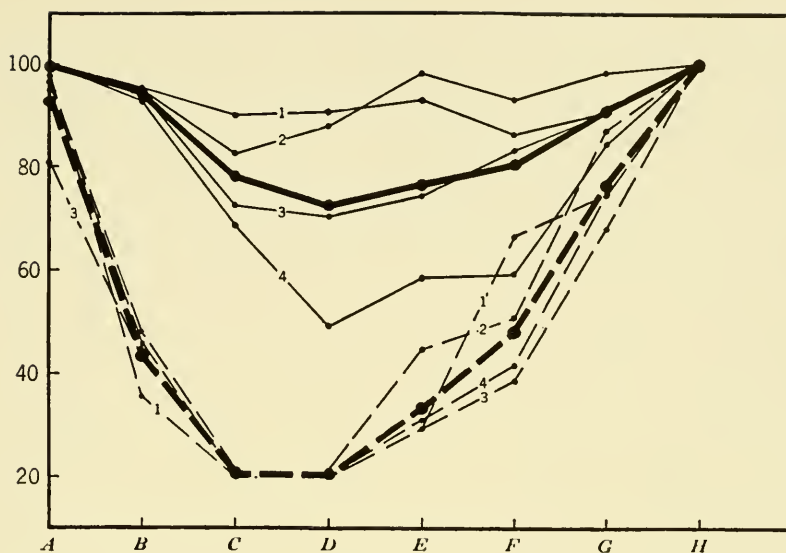


FIG. 70.—Head frequencies of $1/8$ pieces of 16-cm. animals reconstituting in strychnine sulphate $m/450,000$ (continuous lines) and controls (broken lines); corresponding experimental and control lots are indicated by numbers 1-4. Values obtained from combined counts in heavy lines; indices as ordinates, body-levels as abscissae (from F. S. Miller, 1937).



FIG. 71.—Head frequencies of $1/8$ pieces of 16-mm. animals reconstituting in water after 3 days' exposure to $m/300,000$ strychnine sulphate; graphed like Fig. 70 (from F. S. Miller, 1937).

of these, differentially inhibited head forms appear (Abeloos, 1930); in others, an essentially normal head form or no head at all develops on pieces of any length posterior to a certain level.⁸ Apparently, if the initial activation of the head-forming cells occurs in these latter forms it is not inhibited physiologically, even in short pieces. It is perhaps of interest to note that in these species there is more regeneration of new tissue at the anterior ends of pieces than in *Dugesia*; consequently, the developing head and ganglia are isolated to a greater degree from the old nerve cords and so from any nervous factor originating at the posterior cut surface and inhibiting head development. In the dendrocoelids *Procotyla* and *Dendrocoelum* rate of head development decreases from anterior levels of section to a region near the middle of the body; posterior to this level head development has not been observed, irrespective of length of piece or presence of a posterior cut surface, although some new tissue is formed; but posterior ends regenerate at all except extreme anterior levels. Apparently, activation of the cells at the anterior end of the pieces following section decreases more steeply from anterior to posterior levels in these forms than in *Dugesia* and at a certain level becomes insufficient to initiate development of ganglia and head, though still sufficient under the dominance of more anterior levels to develop a posterior end.

SECONDARY DIFFERENTIAL MODIFICATIONS OF THE PLANARIAN HEAD

Not infrequently pieces undergoing reconstitution in water, but with heads inhibited by the physiological factor, show secondary modifications at the anterior end, suggesting a conditioning to, or a recovery from, the earlier inhibition, more probably the latter, since the physiological inhibiting factor is present only temporarily following section. For example, in anophthalmic forms like Figure 63, *I* or *J*, further development often occurs after a week or more, resulting in forms like Figure 72, *A* and *B*. In the case of Figure 72, *A*, the median region of the anterior new tissue has undergone elongation; in Figure 72, *B*, not only elongation but development of a median cephalic lobe and eye has occurred. In this case the outgrowth finally attains a condition permitting development of a teratomorphic head. Development of a normal or teratophthalmic head from these primarily anophthalmic pieces has never been observed.

extremely short pieces, 1/16 or less of the body length, under natural conditions. Animals, apparently of the same species, from another locality several hundred miles from the first showed a head-frequency gradient essentially like that of *D. dorotocephala*, according to data obtained by Professor T. Minoura.

⁸ Sivičkis, 1931a, 1933; Buchanan, 1933.

Heads inhibited by the physiological factor to the less extreme teratomorphic forms such as Figure 63, *E*, often undergo a secondary transformation into forms like Figure 72, *C*, with resorption of the original cephalic lobes and development of new lobes in the normal position and of two eyes, also normally placed. Here the median head region, not represented in the primary development, appears secondarily, and a normal head, except for the primary median eye, results. In other cases all three

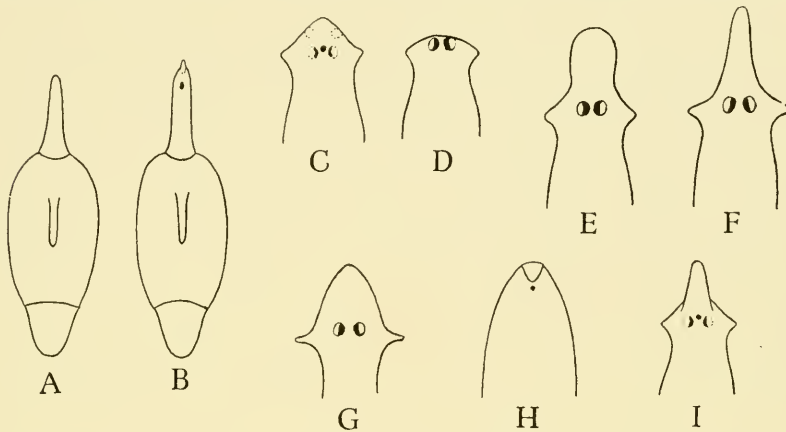


FIG. 72, *A-I*.—Secondary modifications of head form, differential conditioning, and differential recovery in *Dugesia*. *A, B*, differential recovery of anophthalmic heads; *C*, differential recovery of teratomorphic head; *D*, reduction of head and loss of preocular region in low concentrations of alcohol and ether; *E, F*, secondary development of median region under the same conditions that produced the primary effect, *D* (differential conditioning); *G*, approach to normal form after return to water; *H*, reconstitution of head in alcohol, ether, and low temperature; *I*, secondary development of head in alcohol and ether (differential conditioning) and with rise in temperature (differential acceleration) (in part from Child, 1921c).

eyes may be more or less fused, that is, the head does not develop beyond the teratophthalmic condition.

Still more extreme secondary modifications of head form appear with differential conditioning to, and recovery from, effects of external inhibiting agents. When intact animals are placed in low concentrations of ethyl alcohol or ether,⁹ the head decreases in size relatively to the body, and the preocular region often undergoes more or less complete reduction by gradual resorption or by disintegration in the course of 2 or 3 weeks (Fig. 72, *D*). At about this time or somewhat later new growth of the median region may begin in the same concentration in which resorption

⁹ Alcohol 1-1.5 per cent; ether 0.2-0.4 per cent.

or disintegration occurred earlier, and after about a month in the solution head forms like Figure 72, *E* and *F*, have developed. Apparently the median region gradually acquires a greater ability to grow and develop than lateral regions. After such forms are returned to water, the head may gradually approach normal form, but even after two months or more the preocular region is still larger than normal (Fig. 72, *G*).

Very similar modifications of head form occur in reconstitution of heads on pieces under external inhibiting conditions. In ether, alcohol, chloretone, and other anesthetics heads like Figure 72, *H*, appear frequently: in these the growth of new tissue is largely inhibited; but in the course of 8–10 days a single median eye appears, often in the pigmented old tissue. With some further development such heads would doubtless be teratomorphic. After 2 weeks or more in the solution further growth may begin, resulting in heads with elongated median region, and often two new eyes and cephalic lobes appear in normal position (Fig. 72, *I*). Here the median regions not represented in earlier stages gradually develop as conditioning proceeds and become overdeveloped relative to other parts of the head. Essentially the same head forms appear in differential recovery after exposure for a week or two to the same agents. Secondary modifications of the same type have also been obtained in the differential acceleration of development resulting from change to high temperature after conditioning to low. Pieces from a low-temperature stock (conditioned to 3°–5° C.) reconstituting at 4°–8° C. produce little new tissue, and most of them remain acephalic or like Figure 72, *H*, even after 3 months. Brought to room temperature (20°–24° C.) many pieces develop heads with median elongations like Figure 72, *I*, or without median eye. These cases may involve differential conditioning to the higher temperature, as well as differential acceleration of development. The occurrence of these forms with various anesthetics and with change from low to high temperature makes it probable that they, like other modifications of planarian head form, are expressions of differential susceptibility and not specific for any particular agent. Doubtless they can be produced with many other agents which permit relatively rapid differential conditioning or recovery.

THE PLANARIAN HEAD: INTERPRETATIONS AND SUGGESTIONS

It is a point of considerable importance that both the physiological inhibiting factor and the external inhibiting agents give the same inhibition series of head forms in *Dugesia*, a continuous graded series of medio-

lateral differential inhibitions of development. The facts suggest a gradation decreasing from the median region laterally as regards the "physiological level" necessary for initiation of development of the head. That this level involves metabolism seems evident. The action of both physiological and external factors in inhibiting head development apparently consists in preventing activation of the cells concerned to the level required. The inhibition of development involves progressively regions farther lateral as the action of the inhibiting factor increases.

This interpretation will serve for the longer pieces on which no inhibited heads appear under natural conditions. As regards the shorter pieces, however, it appears, at first glance, to be in contradiction to the results of experiments, for agents which inhibit head development differentially in longer pieces with high head frequency usually have the opposite effect on shorter pieces with a naturally low frequency. These agents decrease the mediolateral differential inhibition even though they retard development of the head. These effects of external agents indicate, and experiments on delay of posterior and anterior section, to be presented later (pp. 406-11), provide conclusive evidence, that in the shorter planarian pieces two antagonistic factors are concerned in determining the head form on a particular piece: the one the activation of the cells concerned in head formation; the other the effect, nervous stimulation, or whatever it may be, resulting from section of the nerve cords at the posterior end of the piece. This effect is probably not essentially different from a functional stimulus which tends to maintain the cells as cells of a particular body-level. In order to give rise to a head, they must become free of relations to other parts. Removal of more anterior regions has freed them from relations to those parts; but if nervous stimuli from more posterior regions are sufficient to keep them, to some extent, functional parts of a particular body-level, they do not attain the level of activation necessary for development of a normal head, and the inhibition series of head forms results, according to the effectiveness of the physiological factor. Transverse section of the longitudinal nerve cords with minimum injury of other parts, if within a certain distance of the anterior end of the piece, decreases head frequency; but transverse section of other regions at the same level has little or no effect on frequency (Watanabe, 1935*b*). Also, removal of a short piece half the body width, involving section of one nerve cord a short distance posterior to a level of head regeneration, usually results in asymmetry of the head, the side anterior to the half-section being more or less inhibited (Rulon, 1936*b*).

The shorter the piece at a given body-level and the farther posterior in the anterior zooid the level of origin, the more effective is the physiological inhibiting factor. The length of piece at which inhibition of the head begins to appear increases from anterior to posterior levels of the anterior zooid. At the anterior level the physiological factor is effective only in very short pieces. The intensity or rate of activation of the head-forming cells and the rate of head development decrease from anterior to posterior levels (p. 43), and it may be that the nervous effect of posterior section is better transmitted in the anterior direction at more posterior levels. Either or both of these conditions may determine the greater effectiveness at a given distance and effectiveness at a greater distance of the physiological inhibiting factor at more posterior levels.

In any lot of similar pieces head frequency depends on the relation between the condition of the cells directly concerned in head formation and the physiological factor preventing those cells from undergoing the change in condition necessary for complete head development. The physiological factor has been called an "inhibiting factor" because it inhibits head development differentially, but it appears actually to be a factor which tends to maintain the cells as functional parts of a certain region of the body. In order to give rise to a head, the cells must become free from functional relation to other parts of the body; and the physiological differential inhibition of head development represents all degrees of inability to attain that freedom, because the nervous stimulation resulting from posterior section tends to maintain another sort of cellular behavior. The parts of the head which require the highest level of activation for initiation of their development are first and most inhibited; these are the median head regions. With lesser degrees of freedom more lateral regions are reduced or prevented from developing, until, when the cells are completely dominated by the physiological effect of posterior section, they do not react at all to the absence of regions anterior to their level, and acephalic forms result. According to length of piece, level of origin, physiological condition of parent animal, and nature and concentration of external agent and period of exposure, the degree of differential inhibition of head development varies in definite, orderly ways. Experimental control of head frequency is possible by controlling length of piece and level of origin and condition of parent animal and also by external inhibiting and accelerating agents. All these methods of control are merely ways of controlling and altering the balance between the two antagonistic factors concerned in determining the degree of differential inhibition of head development.

Differences in effect of the various external agents on head frequency depend on their relative effect on the two factors. These which inhibit directly the activation of the head-forming cells decrease head frequency even in long pieces in which the heads are not physiologically inhibited. Those which inhibit nervous activity are effective in increasing head frequency in short and posterior pieces in which it is physiologically inhibited, but they may also inhibit the head-forming cells directly and so decrease head frequency in long and anterior pieces in which it is not physiologically inhibited. Cyanide, for example, inhibits both factors and may decrease or increase head frequency according to length of piece, level of body, period of exposure, and concentration; but results can be controlled and, with sufficient experimental background, predicted. Since the physiological factor inhibiting head development is effective only temporarily following section, temporary exposure to cyanide for a day or two after section is most effective in increasing head frequency because, after return to water, the head-forming cells recover more or less completely and are not longer inhibited by the nervous stimulation resulting from posterior section. Exposure to cyanide during the whole period of reconstitution is most effective in decreasing head frequency, because under these conditions its direct action on the head-forming cells is continuous. The anesthetics used in controlling head frequency act more or less like cyanide but are more effective in increasing frequency by inhibiting the nervous stimulation than in decreasing it by direct action on the head-forming cells. Carbon dioxide, hydrogen ion, organic acids, and strychnine inhibit the nervous factor but have relatively little effect, in the concentrations used, on the head-forming cells; consequently, they are much more effective in increasing head frequency in short and posterior pieces than in decreasing it in long and anterior pieces. Temperature, on the other hand, apparently affects chiefly the head-forming cells: a rise in temperature increases, a fall decreases, head frequency. Caffein apparently may serve as an accelerating or a depressing agent according to concentration and exposure period; consequently, it may either increase or decrease head frequency in pieces of the same sort.

Differential conditioning to low concentrations of external agents and differential recovery may also alter head frequency secondarily: teratomorphic heads may become normal, except for the original median eye, and anophthalmic forms may become teratomorphic. Also, a series of differentially modified head forms, with overdevelopment instead of inhibition of the median region, occurs. These modifications are, of course,

not direct effects of physiological or external factors inhibiting head development but represent secondary differential reactions of the developing head region following a primary inhibition.

There is, at present, no evidence of specificity in the differential modifications of head form. It was noted above that they do not concern any particular organ or region of the head but involve any and all parts. The differential inhibitions of head form constitute a continuous graded series ranging from slight inhibition of the median region to complete inhibition of the whole head. The inhibiting factor, whether physiological or an external agent, acts, of course, on the earlier stages of head regeneration before the various organs are present as localized differentiations. The secondary modifications, although appearing under a rather narrowly limited range of conditions, evidently also constitute a continuous graded series. The appearance of the same series of forms with physiological factors and with many external agents suggests that the effects of all these different factors on the primary pattern of the planarian head must be essentially similar. In other words, the only possible conclusion, in view of all the evidence, seems to be that the mediolateral pattern which is differentially modified must consist primarily in a quantitative gradient or differential. If specific differences are present in the cells from which the head develops, they are not concerned in the differential modifications of form and proportion. However, the differentiations which appear in the modified heads suggest that the quantitative gradient provides the physiological basis for differentiation, and the differential susceptibility of different levels of this gradient to physiological and external factors determines not only form and proportions of the modified heads but the differentiations which appear in them.

Absence of physiological differential inhibition of head development in certain triclads may be due to one or more of several factors: activation of the head-forming cells may be so intense that the nervous factor cannot prevent it, or the nervous factor may be slight or less readily transmitted anteriorly in some species than in others; in some species head regeneration is more completely isolated from the old nerve cords by regenerating tissue posterior to it; in some the head develops so slowly that the transitory nervous effect of posterior section may disappear before it can be effective in inhibiting head development. Only further experiment with the various forms can throw light on these points.

CHAPTER VI

DIFFERENTIAL MODIFICATION OF DEVELOPMENT: ECHINODERMS

BECAUSE of its plasticity under experimental conditions, echinoderm development in its early stages is exceptionally interesting material for investigation of differential modification of development. The alterations of form and proportions and the changes in localization of parts resulting from exposure of the whole developing organism to experimental environments are, to a high degree, definite in character and experimentally reproducible. They are not simply "abnormal" or teratological forms to be described as curious or mysterious anomalies of development, but alterations in definite ways under controllable conditions; as such, they constitute a highly significant body of evidence bearing on problems of the physiology of developmental pattern. At present little more than a beginning has been made in this field of physiological analysis, but results obtained speak for themselves and promise much of interest to further investigation. Thus far, only echinoids and asteroids have been used as material.¹

ECHINOIDS

FORM AND PROPORTIONS UNDER NATURAL CONDITIONS

Echinoid development to the pluteus larva follows, in general, the same course in different groups and species with only minor differences in form and proportions, skeletal pattern, etc. Cleavage, blastula, and gastrula stages are figured in chapter iv (Figs. 44-46, and 49, *A*, *B*). In Figure 73 the gastrula (*A*), the prepluteus (*B*, *C*), the pluteus (*D*, *E*) of the sand dollar *Dendraster excentricus*, and the pluteus of the sea urchin *Arbacia punctulata* (*F*, *G*) are shown.

¹ In the following account figures are used extensively as more effective than any amount of description. Most of the figures are based on ocular micrometer measurements of the dimensions of living individuals, supplemented by sketches of the same individuals. Form and proportions are shown as exactly as possible, but various details of structure are often omitted or diagrammatically indicated, particularly mesenchyme and skeleton. All figures of a group are on the same scale. For data concerning concentrations of agents and exposure periods, supplementing those given in the legends, see Appendix VIII (p. 747).

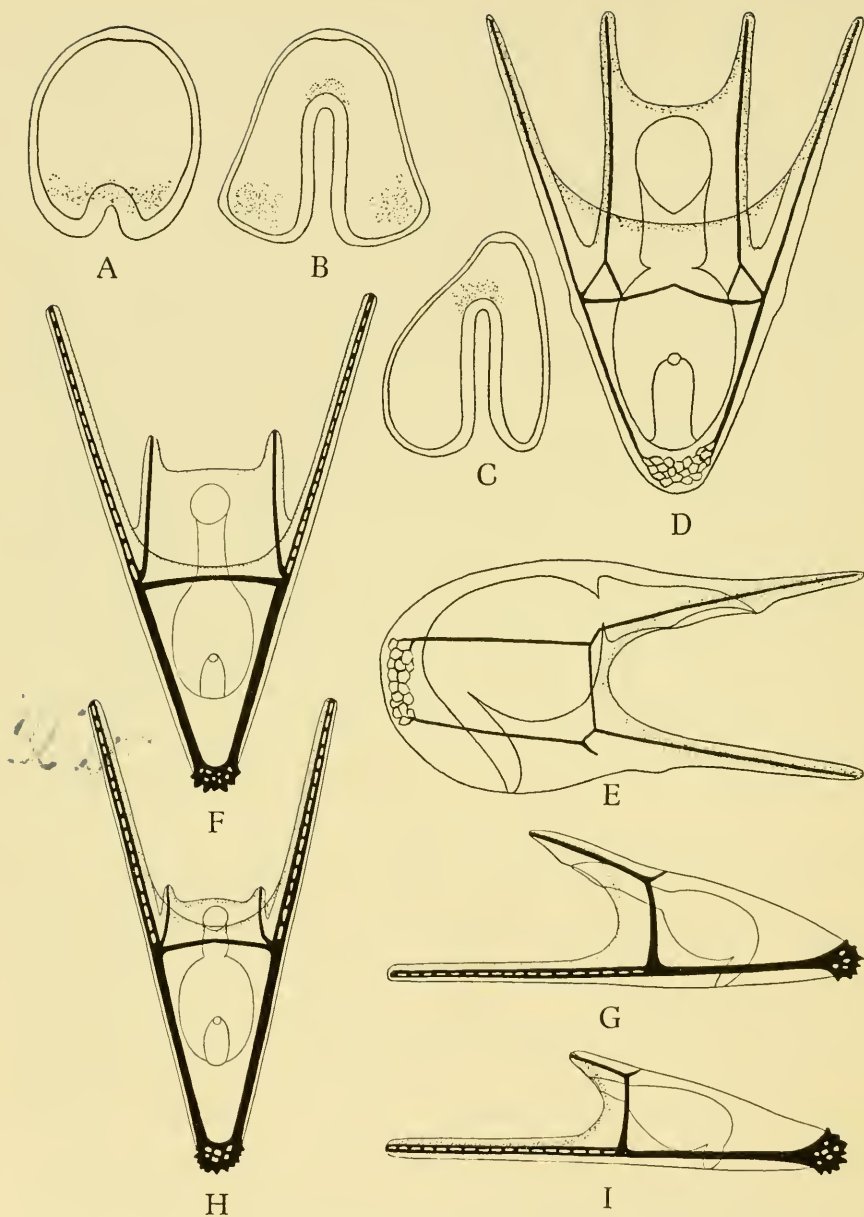


FIG. 73, A-I.—Echinoid development under natural and slightly inhibiting conditions. A-E, development of *Dendraster excentricus* from gastrula to pluteus under natural conditions; F, G, pluteus of *Arbacia punctulata*; H, I, slight differential inhibition of *Arbacia* pluteus by development in KCN m/100,000 (F-I from Child, 1916b).

DIFFERENTIAL INHIBITION

In slight degrees of differential inhibition at early stages the oral lobe is most inhibited, the angle between the anal arms (brachial angle) is slightly decreased, and the foregut or esophagus is usually small (Fig. 73, *H*, *I*); that is, apical and mid-ventral ectodermal regions are most in-

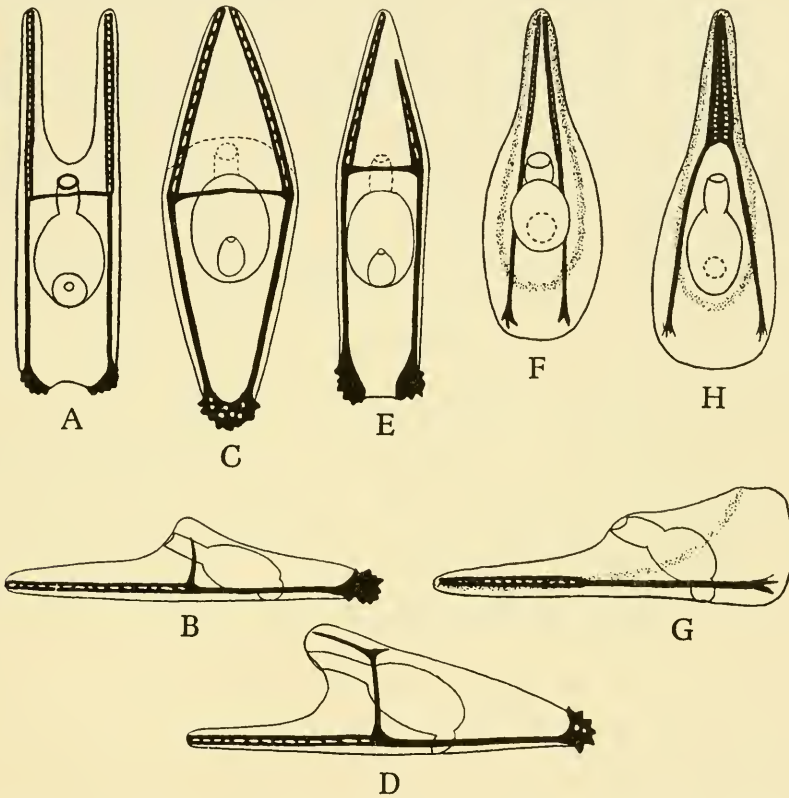


FIG. 74, *A-H*.—Differential inhibition in *Arbacia*. *A-E*, KCN; *F-H*, CuSO_4 and LiCl (*A-E* from Child, 1916*d*).

hibited. With greater inhibition there is further reduction of the oral lobe, often to complete absence, and further decrease of brachial angle to parallel, fused, or single median arms with skeletal rods parallel or even converging toward the tips (Fig. 74). In Figure 74, *A-E* are cyanide material, *F-H*, CuSO_4 and LiCl , with return to water after exposure long enough so that the differential inhibition persists, although development may continue. With such procedure the differential inhibition has become

irreversible, at least up to the most advanced stage attained. Similar forms appear in acid sea water, sea water plus ammonia, ethyl alcohol, hypotonic sea water, $MgCl_2$, etc., with certain concentrations and exposure periods. Unfertilized eggs in KCN, m/100 up to a few hours, then fertilized and developing in water, give similar forms. The forms of Figure 74 show not only an apicobasal but also a ventrodorsal and a mediolateral

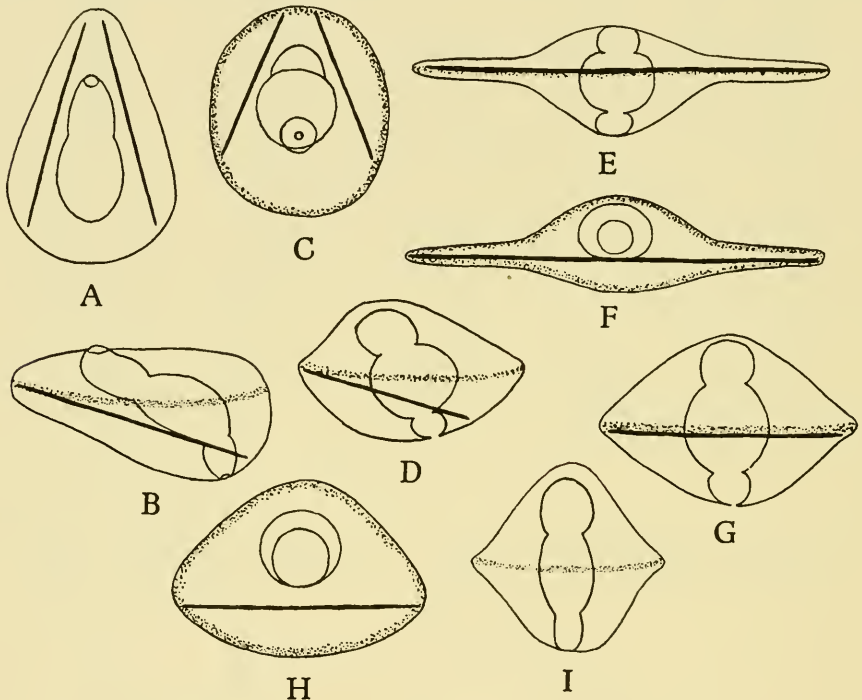


FIG. 75, A-I.—Differential inhibition in *Arbacia*, showing various degrees of obliteration of the original ventrodorsality and the resulting bilaterality. A-D, ventrodorsality still evident; E-H, bilaterality perhaps secondary and determined by transverse orientation and elongation of skeletal rod; I, completely radial form without skeleton. Ciliated band shaded.

differential inhibition. It seems evident that decrease of brachial angle, approximation and fusion of arms, and development of a single median arm result from different degrees of ventral inhibition, decreasing from the median region laterally and quite similar in principle to the mediolateral differential inhibition of planarian head development (pp. 177-81).

A series of forms with further degrees of differential inhibition and decrease of axiate pattern is shown in Figure 75. These forms occur most frequently with long-time exposures (e.g., 36 hours or more) from early

stages to LiCl in concentrations slightly above the range in which differential tolerance or conditioning is possible. The long period of exposure makes the inhibition practically irreversible within the developmental period, though there is some further development after return to water and perhaps slight differential recovery, but differential inhibition is evidently predominant. These forms, however, are not specific for lithium but have been seen with other agents having a high differential action—for example, CuSO_4 , HgCl_2 , and very similar forms, though with differentiation more inhibited, are produced by KCN. In the individual of Figure 75, *A* and *B*, there is evidently extreme apical inhibition, the oral lobe being completely absent; the skeletal rods converge toward what is apparently the ventral side, but arms do not develop, and the ciliated band is apparently more or less transverse. Figure 75, *C* and *D*, shows a more extreme case of this type, with further approach to radial form but still with some evidence of ventrodorsality in position of the skeletal rods. In lots subjected to the same conditions forms like *E-I* of Figure 75, also occur; in these forms apicobasal pattern is almost completely obliterated and only a single skeletal rod is present, apparently transverse and in the same plane as the ciliated band, suggesting a possible relation. But whether, or to what extent, position of arms 180° apart, when they develop, is determined by prospective arm areas in the ectoderm or merely by elongation of the skeletal rod is uncertain. Elongation of a rod can induce development of an arm or armlike outgrowth in other than the position of the original arm area. In forms with excess of skeleton supernumerary arms are often formed, three-, four-, and five-armed forms resulting; and in the apparently anaxiate ectoderm of extreme exogastrulae a skeletal rod may induce a short armlike outgrowth in various positions, even at what was originally the apical pole. It seems possible, therefore, that the arms of forms like *E* and *F* of Figure 75 result from skeletal elongation. If this is the case, they may be without definite relation to the original ventrodorsality and constitute a bilaterality independent of it. With less elongation of the skeletal rod bilaterality is less evident (Fig. 75, *G*, *H*). That the original ventrodorsality and the resulting bilaterality may be completely obliterated is suggested by absence of stomodeum and by completely radial form of entoderm. These forms are very different from the wide-angled forms resulting from secondary modifications described in the following section and appear only under rather extreme inhibiting conditions, but they probably do involve some slight recovery, for development of ciliated band, skeleton, and arms occurs only after

return to water. In the absence of skeletal development completely radial individuals with apparently transverse ciliated band are frequent in the same experimental lots (Fig. 75, *I*). This series of forms has been obtained with *Arbacia*, *Strongylocentrotus*, and *Dendraster*.

As degree of inhibition increases, there is progressively less evidence of axiate pattern, the skeleton is represented by a few spicules or does not develop, and mesenchyme cells are scattered instead of being localized bilaterally in the regions of arm development. The particular form of larva resulting depends largely on the stage at which exposure begins and the rate at which inhibition occurs in relation to rate of development. With relatively rapid inhibition, beginning at the two-cell stage and return to water before the increase in susceptibility of entoderm associated with gastrulation, ectodermal development may attain slight ventrodorsality (Fig. 76, *A*) or be completely radial, and more or less regional entodermal differentiation may occur. Although these forms probably represent a slight degree of differential recovery, they are mentioned here because they represent chiefly considerable degrees of differential inhibition. Other somewhat similar forms are described below under differential recovery. With sufficient inhibition involving the early gastrula entoderm is inhibited, develops incompletely, or may become spherical, separate completely from the closed blastopore, and lie free in the blastocoel. Ectoderm may show slight polarity or be completely anaxiate (Fig. 76, *B*, *C*). Forms of this sort, returned to water, may live for 2 weeks or more. Completely anaxiate forms like Figure 76, *C*, do not show definitely directed locomotion but roll about in all directions. With still more extreme inhibition in early blastula stages more or less cytolysis and disintegration progress basipetally from the apical pole. With return to water at the proper time the disintegration is arrested, the cells come together apically, and invagination of entoderm may take place (Fig. 76, *D*, *E*). Some of these forms remain completely anaxiate; others show slight differential recovery (pp. 208-10). With these relatively extreme inhibiting conditions continuing from early stages to the stage when entodermal susceptibility increases, the entoderm tends to lose its epithelial character and become a solid cell mass, from the surface of which cells dissociate. This dissociation may begin either without invagination or during its early stages (Fig. 76, *F-I*). Cells may also dissociate from regions apical to the prospective entoderm, in some cases from all levels, as in *Phialidium* (pp. 167-69). In many of these cases, particularly with LiCl but also with other agents, there is entodermization of prospective ectoderm; that

is, a part, or in extreme cases all, of the prospective ectoderm becomes entoderm. If such forms are able to develop further after return to water, they become exogastrulae with small or no ectoderm and large external entoderm. With exposure to LiCl beginning in the later blastula stages,

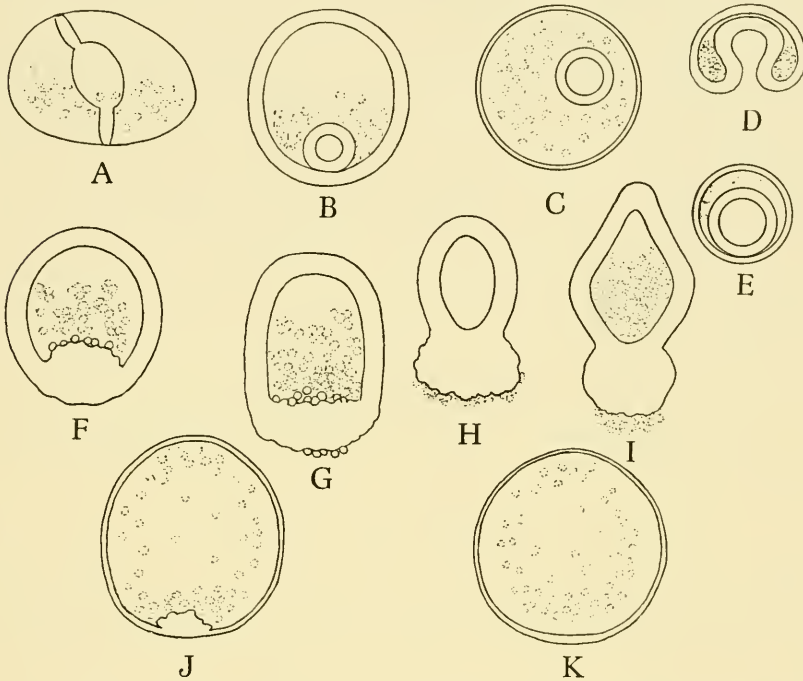


FIG. 76, A-K.—Differential inhibition. A-E, *Arbacia*, KCN, ethyl alcohol, etc. A, slight axiation remaining; B, ventrodorsality not evident, apparently slight polarity; C, completely anaxiate; D, E, partial differential death, apical region of early blastula killed, development of basal region, but E anaxiate; F-I, *Dendraster*, extreme differential inhibition in high concentrations of LiCl (e.g., m/20) continuing from two-cell stage to stage when susceptibility of prospective entoderm becomes higher than that of ectoderm; prospective entoderm and more or less of the entodermized ectoderm becoming a solid cell mass from which cells dissociate externally or internally or both; J, K, *Strongylocentrotus franciscanus*, showing inhibition of entoderm with exposure to LiCl (m/40) beginning in later blastula stages (A-D, after Child, 1916d; H, I, from Child, 1940; J, K, from Child, 1936b).

about the time of increase in susceptibility of the entoderm, not only may further entodermal development be inhibited (Fig. 76, J) but entoderm may decrease in extent and thickness so that more or less of it becomes indistinguishable from ectoderm (Fig. 76, K). Forms of this type suggest that some of the prospective entoderm has been ectodermized. If this is

the case, it appears that the same inhibiting agent may entodermize prospective ectoderm in early stages, when it represents higher gradient-levels than entoderm, and ectodermize prospective entoderm in later stages, when it has attained a higher gradient-level than ectoderm. Further examples of this differential inhibition will be given in the section on exogastrulation.

The so-called "sterroblastula," appearing so frequently under widely varied inhibiting conditions and often in pathological lots of eggs without external inhibition, is a blastula in form with blastocoel more or less completely filled with cells. It represents a differential inhibition with dissociation of cells from the entodermal region and perhaps also from other parts of the wall. Apparently, all that is necessary to produce sterroblastulae is a sufficient degree of inhibition. In general, the developmental modifications resulting from differential inhibition differ with concentration or intensity of agent, with exposure period, and with stage when exposure begins; but there is no conclusive evidence of specific regional effects of particular agents: the differences appear rather to be indicative of quantitative factors in physiological condition and their changes in the course of development than of specific factors.

SECONDARY MODIFICATIONS: DIFFERENTIAL TOLERANCE, CONDITIONING, AND RECOVERY

These secondary modifications of development follow an initial inhibition; they are much greater and occur much more rapidly with some agents than with others. With cyanide, for example, they occur slowly and are not extreme; with ethyl alcohol, acidified sea water, hypotonic sea water, and various other agents they appear earlier and may be extreme; with CuSO_4 and MgCl_2 they appear somewhat more slowly but may still be great.²

With slight inhibition in early stages the first evidence of differential tolerance or conditioning is relative elongation and enlargement of the apical region; this may appear in the gastrula with slight inhibition by agents such as ethyl alcohol, acidified or hypotonic sea water, and various others (Fig. 77, *A, B*), but with many agents it becomes evident somewhat later. In the prepluteus the oral lobe becomes relatively large and long (Fig. 77, *C*), and the resulting pluteus has a large oral lobe and a wide brachial angle up to 180° , a relatively large ventral, and a small dorsal

² *Arbacia* has been the chief material in these experiments, but data on *Strongylocentrotus* and *Dendraster* are sufficient to show that the secondary modifications in these species are essentially similar.

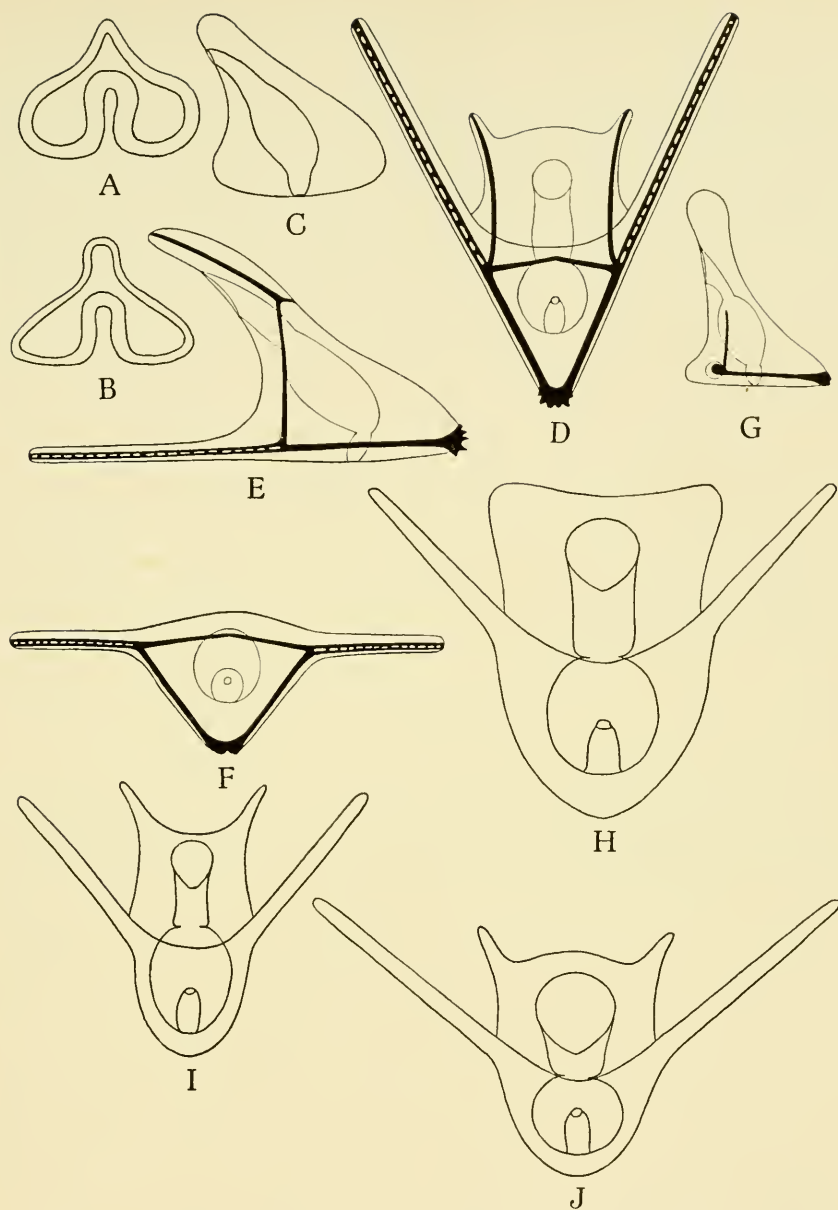


FIG. 77, A-J.—Secondary modifications resulting from differential tolerance or conditioning and differential recovery. A-G, *Arbacia*; H-I, *Dendraster*. A, B, secondary modification of apical region of gastrula in ethyl alcohol 2 per cent; C, prepluteus with elongated apical region (oral lobe), a secondary modification characteristic with various agents; D-G, two plutei in anal (D, F) and lateral (E, G) outline with oral lobe and ventral regions relatively enlarged (characteristic with various agents); H, differential tolerance or conditioning with LiCl m/90; I, J, differential recovery after $4\frac{3}{4}$ hr. in LiCl m/30 from early blastula.

region. Comparison of the *Arbacia* plutei of Figure 77, *D-G*, with those developing under natural conditions (Fig. 73, *D, E*) shows the alteration in proportions. Similar modifications with low concentrations of LiCl appear in *Dendraster*: Figure 77, *H*, is an example of differential tolerance or conditioning to lithium, and *I* and *J* of Figure 77 are cases of differential recovery from lithium: pluteus form under natural conditions is shown in Figure 73, *F*. In all these wide-angled plutei the ciliated band has the usual relation to oral lobe and arms, and the characteristic skeletal pattern develops but with alterations in proportions corresponding to those of other parts.

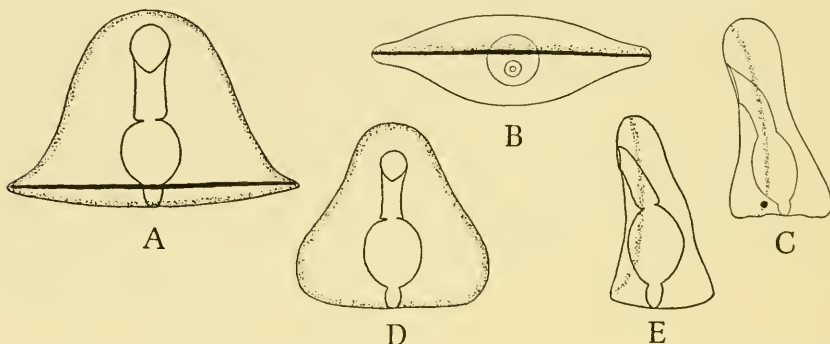


FIG. 78, *A-E*.—More extreme secondary modifications in *Arbacia* with conditioning to, and recovery from, alcohol, acid sea water, CuSO_4 , HgCl_2 , etc. *A-C*, ventral, anal, and lateral outlines of an individual; *D, E*, ventral and lateral outlines of another individual without skeleton (after Child, 1916b).

With somewhat greater degrees of inhibition the secondary modifications are greater. Characteristic forms of *Arbacia* are outlined in Figure 78. These larvae may be said to consist largely of oral lobe; this is much enlarged and elongated relatively to other parts but still shows inhibition in later differentiation. The larvae are flattened ventrodorsally, and arms are more or less inhibited or absent. The skeleton may be limited to a single rod, as in Figure 78, *A-C*, or the brachial rods of the two sides may be separate; and in some animals there are other spicules or the skeleton may be entirely absent (Fig. 78, *D, E*). Here the secondary modifications of ectoderm are evidently apicoventral and decrease basipetally. The usually large foregut suggests secondary modification in the apical entodermal region, the high end of the entodermal gradient. The skeleton is more or less inhibited or absent, probably because activation

of mesenchyme at the time of immigration is more or less inhibited; and before tolerance, conditioning, or recovery occurs, it is too late for normal skeletal development. These forms also are not specific for any particular agent, but their appearance in a given species depends on concentration or intensity, developmental stage, and period of exposure. Although the skeleton may consist of a single rod transversely oriented, these forms are of quite different origin from those of Figure 75, *E-H*, which are results

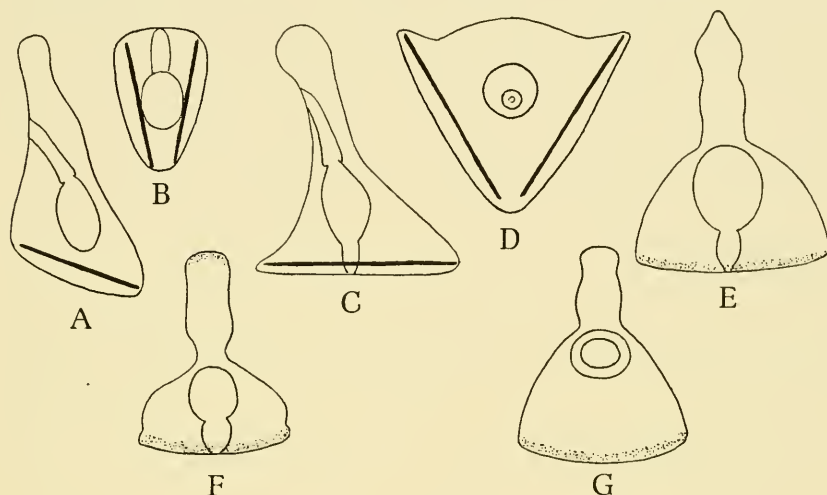


FIG. 79, *A-G*.—Secondary modifications in *Arbacia*. *A, B*, lateral and basal outlines, continuous in alcohol, 1.5 per cent, with apical region secondarily modified, basal region differentially inhibited; *C, D*, lateral and basal outlines, CuSO_4 $m/2,500,000$, continuous; *E-G*, secondary modifications in apical region only, ventrodorsality obliterated, CuSO_4 $m/2,500,000$, continuous.

of relatively extreme inhibition with almost complete or complete obliteration of ventrodorsality and decrease of polarity.

The most extreme secondary modifications resulting from differential conditioning with continuous exposure to low concentrations of agent have been obtained with ethyl alcohol and CuSO_4 . In Figure 79, *A* and *B* (alcohol, 1.5 per cent) and *C* and *D* (CuSO_4 $m/2,500,000$) are examples. In these the elongation and enlargement of the apical region is secondary, early development being inhibited. Inhibition persists in the basal region, arms being completely absent and the skeletal rods oriented at less than the usual angle (*B*), or arms are present and greatly inhibited, but the angle of the skeletal rods is slightly wider than normal, suggesting slight

secondary modification ventrally (*D*). In somewhat more inhibited individuals secondary modification may be limited to the apical region. In Figure 79, *E*, *F*, and *G*, relatively extreme examples are shown of a type very common with certain ranges of concentration and exposure for all agents used. Ventrodorsality is obliterated in the body, and a transverse ciliated band surrounds the basal region.³ This altered position of the band is characteristic of these forms and also appears in many exogastrulae (pp. 224-25). Apparently the more apical levels of the ventral side are so much inhibited that the band cannot develop there; but with secondary modification in the less inhibited basal region and the altered relations of regions it differentiates there, in part with localization different from the normal. However, the tip of the secondary apical outgrowth often develops the specialized ciliated epithelium characteristic of the band.

Inhibition beginning just before, or at the earliest stages of, entodermal invagination and within the range permitting some degree of secondary modification gives results of interest. At these stages entoderm and mesenchyme have undergone or are undergoing increase in susceptibility and rate of dye reduction. Conditioning or recovery occurs in the apical region, and entoderm and mesenchyme are more or less inhibited. These modifications appear with the various agents used, even in a certain range of concentrations of LiCl (Fig. 80). In earlier stages lithium may entodermize prospective ectoderm, as already noted, and so increase entoderm at the expense of ectoderm; but at this stage entoderm has become so highly susceptible that it is inhibited, while the apical ectoderm, at first inhibited, gradually becomes thicker and elongates. The lithium forms of Figure 80 do not usually develop much farther with continued exposure. No skeleton is formed, the mesenchyme, not shown in Figure 80, remaining scattered or irregularly massed in the blastocoel. Incidentally it may be noted that these forms with relative enlargement of the apical region suggest an approach to asteroid larval form with its large preoral region (Child, 1938). The apicobasal dye-reduction gradient in early stages of the starfish appears to be steeper than that in the sea urchin (Child, 1936a), and these differential modifications involve an increase in steepness of the gradient, at least at the more apical levels.

Lesser degrees of differential tolerance or conditioning are outlined in Figure 81, and of differential recovery after temporary exposure in

³ In some individuals the band develops as two separated parts symmetrically localized on the two sides, indicating that the bilateral features of ventrodorsality have not been completely obliterated.

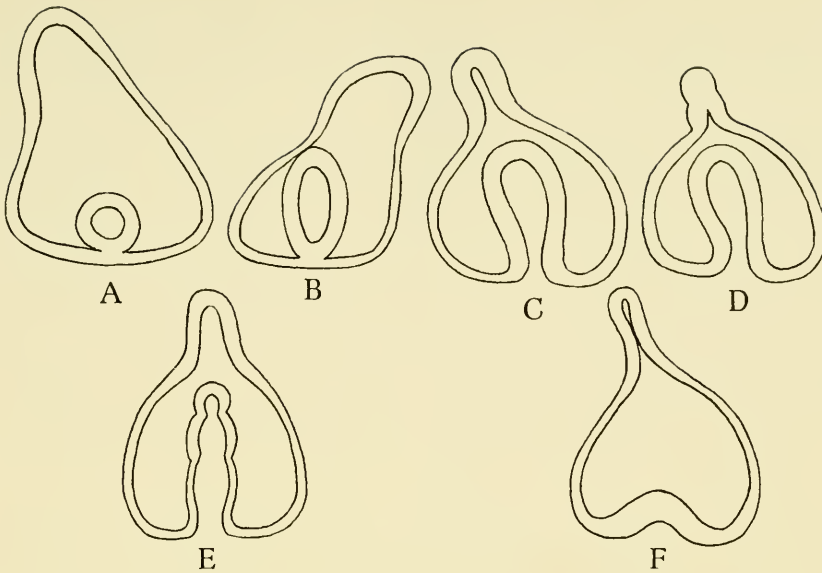


FIG. 80.—Secondary modifications of gastrula of *Dendraster* with 40 hr. of exposure to LiCl m/60 from beginning of gastrulation; apical region secondarily elongated, entoderm inhibited (from Child, 1938).

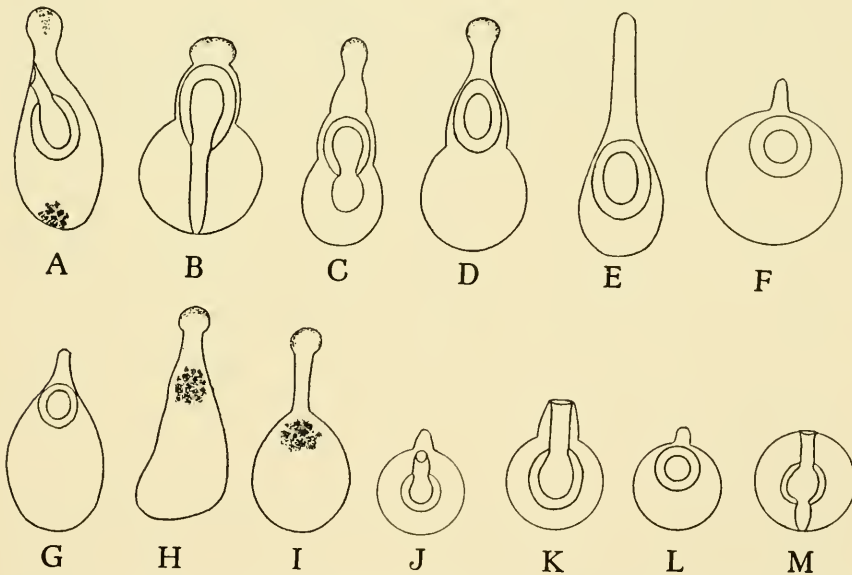


FIG. 81.—Lesser degrees of secondary modification in *Arbacia* limited to apical region with continuous exposure to various agents (from Child, 1916d).

Figure 82. The two series are very similar in that the secondary modifications are limited to the apical region. Position of the mouth at one side

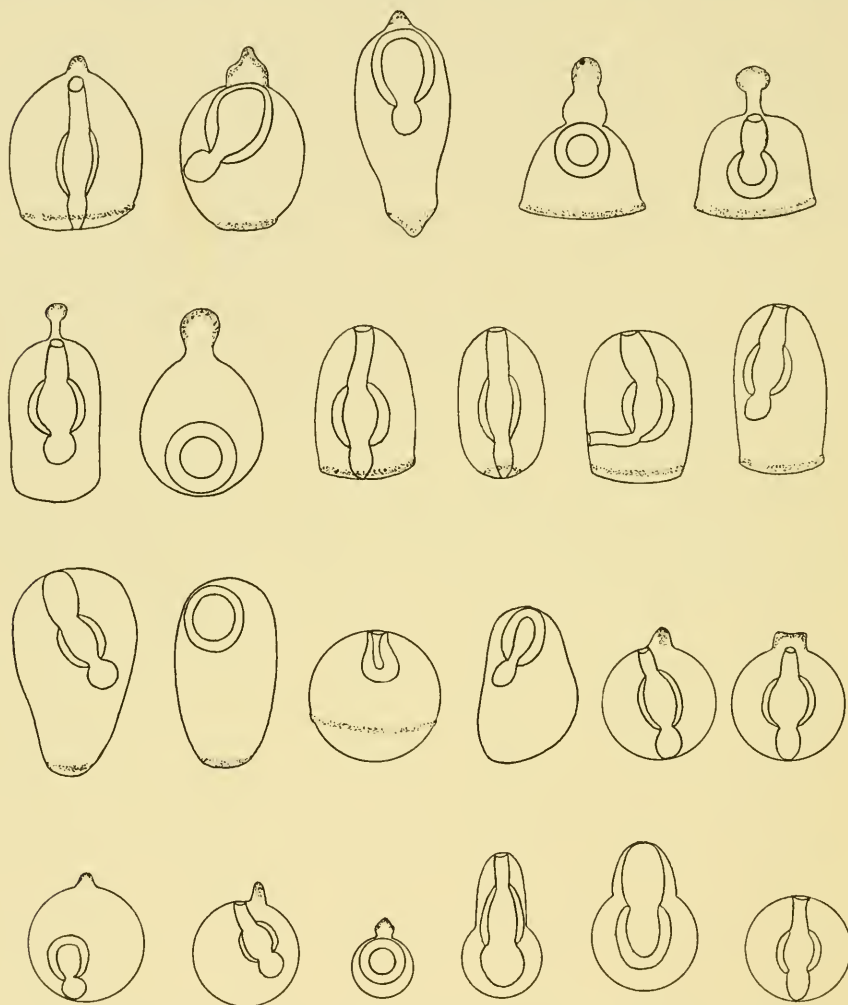


FIG. 82.—Slight differential recoveries in *Arabacia* after relatively extreme inhibition by various agents (from Child, 1916*d*).

in some, presumably the ventral side, of the apical outgrowths suggests that a trace of ventrodorsality is still present apically; but there is no evidence of it elsewhere. In others, however, the mouth is apparently apical, suggesting possible induction of a mouth by the entoderm. Degree

of development of entoderm is highly variable: in some, all three regions are present; in others, two; while in still others it is a spherical vesicle. Very often it separates from the region of the blastopore, of which no trace remains, and usually it comes to lie near the secondarily modified apical region. The secondary apical modification consists in outgrowth, often with development of ciliated band epithelium, in development of a mouth, or occasionally only in apparent attachment of entoderm to the apical ectoderm. If a ciliated band develops, it is basal. The blastocoel contains cells in varying number (not figured), the mesenchyme, and in many individuals cells dissociated from prospective entoderm, but no skeleton develops. Figures 81 and 82 represent the most advanced stages of these forms, but they often live longer without further development than fully developed plutei from the same lots of eggs.⁴

ASTEROIDS

Differential modifications of asteroid development show the same relations as those of echinoids to the gradient pattern indicated by other methods. In differential inhibition apical and ventral regions of ectoderm and apical region of entoderm are most inhibited, and in the secondary modifications these regions become relatively large.

EARLY LARVAL FORM UNDER NATURAL CONDITIONS

The gastrula of the starfish *Patiria miniata* was outlined in Figure 49, C-E, of chapter iv; a later stage is shown in Figure 83; development of *Asterias* is similar. As a basis for consideration of the differential modifications it is important to note the following points of difference between asteroid and echinoid development: first, in the asteroid the mesenchyme does not immigrate before gastrulation but is first formed from the apical entoderm after invagination is completed and increase in rate of dye reduction in the enlarged apical entoderm has occurred (Child, 1936b); second, the invaginated asteroid archenteron extends only about halfway from the basal to the apical pole; third, instead of becoming a flattened oral lobe, as in echinoids, the apical region of the starfish develops into a

⁴ These types of secondary modification have been obtained with alcohol, acid and hypotonic sea water, CuSO₄, HgCl₂, ultra-violet radiation, and visible light with sensitization by eosin. Differential tolerance or conditioning to KCN is slight, but differential recovery gives the whole series of forms after certain ranges of concentration. Similar secondary ectodermal modifications occur with LiCl, but very commonly in association with some degree of exogastrulation.

rounded preoral region, empty except for mesenchyme; fourth, two ciliated bands extend obliquely around the body, as indicated in Figure 83.

DIFFERENTIAL INHIBITION

Experiment on asteroids is less extensive than on echinoids, but a number of agents have been used to produce modifications.⁵ Not the slightest evidence has appeared in more than a hundred experimental lots of specific relation between any of these agents and any particular modification, not even between lithium, exogastrulation, and entodermization of pro-

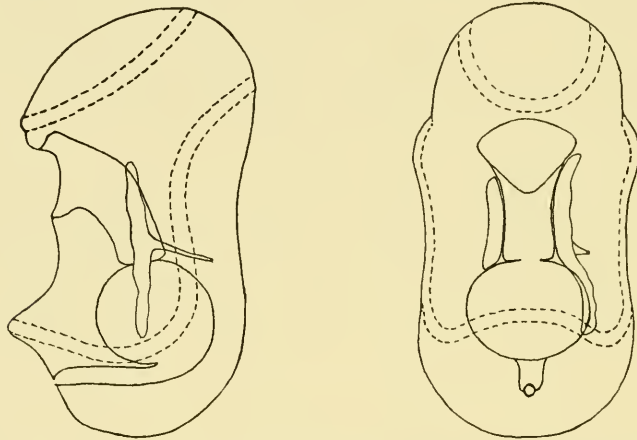


FIG. 83.—Lateral and ventral outlines of larva of *Patiria miniata*; ciliated bands indicated by broken lines (from Child, 1938).

spective ectoderm. Eggs from animals kept in laboratory aquaria supplied with flowing sea water for 9 or 10 days before use develop differentially inhibited forms identical with those obtained with cyanide and lithium, etc. With exposure to the agent in inhibiting concentrations at beginning or early cleavage inhibition at first decreases basipetally, as in echinoids. In differentially inhibited blastulae the polar axis is short and the apical ectoderm thicker than normal. If the inhibition is not too extreme to permit gastrulation, these blastulae, returned to water shortly before gastrulation, or gastrulating before return, form gastrulae like those of Figure 84. In all of these ectoderm is more inhibited than ento-

⁵ The following agents have been used: LiCl and KCN in many concentrations; hypotonic sea water; the dyes neutral red, Janus green, and Nile blue sulphate; crowded conditions; and sea water at pH 7, with CO₂ probably the effective agent.

derm, which is relatively or absolutely "too large." In the first gastrula of Figure 84, 24 hours in water after LiCl, the thin apical ectoderm and the extremely large apical entoderm suggest some degree of differential recovery.

As invagination progresses, entoderm becomes more susceptible than ectoderm, at least in its more apical levels, and when sufficiently inhibited it begins to lose its epithelial character and to undergo dissociation. The lesser degrees of this change resemble immigration of mesenchyme in echinoids, but dissociation may involve the whole entoderm

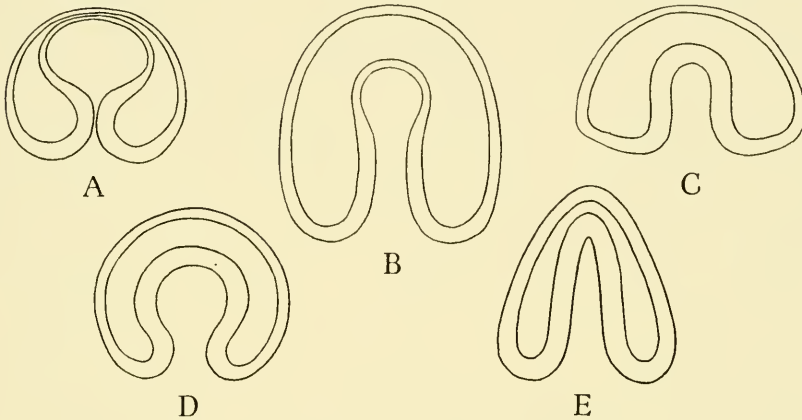


FIG. 84, A-E.—Differentially inhibited gastrulae of *Patiria*. A, LiCl m/25, 11 hr. from sixty-four cells, 24 hr. water; B, LiCl m/30, 14 hr. from sixty-four cells, 26 hr. water; C, LiCl m/25, 19½ hr. from thirty-two cells, 4 days water; D, LiCl m/25, 11 hr. from sixty-four cells, 24 hr. water; E, KCN m/200,000 from two cells; perhaps some differential recovery in apical archenteron of A and B.

(Fig. 85). In the case of Figure 85, A, exposure to a very high concentration of LiCl (m/10) began just before gastrulation; some invagination occurred before the agent became fully effective, but gradually the apical entoderm began to lose epithelial order and to dissociate, and the change progressed basipetally but the ectoderm remained intact. This was the characteristic effect in the experimental lot. In Figure 85, B, from another lithium lot, the invaginated entoderm has become a solid cell mass and is dissociating. These results, characteristic of lithium in sufficient concentration, with exposure beginning just before gastrulation (A) or continuing into that stage, indicate the change in condition of the pregastrula associated with gastrulation. As in echinoids, lithium in early pregastru-

lar stages may entodermize prospective ectoderm, but later it inhibits and dissociates entoderm; the increase in entodermal susceptibility apparently occurs at a somewhat later stage in asteroids than in echinoids, as does the increase in rate of dye reduction (pp. 134-37). Inhibition beginning in early stages may prevent gastrulation, but with long-continued exposure dissociation into the blastocoel of entodermal cells may take place without invagination; apparently, increase of entodermal susceptibility is possible without invagination.

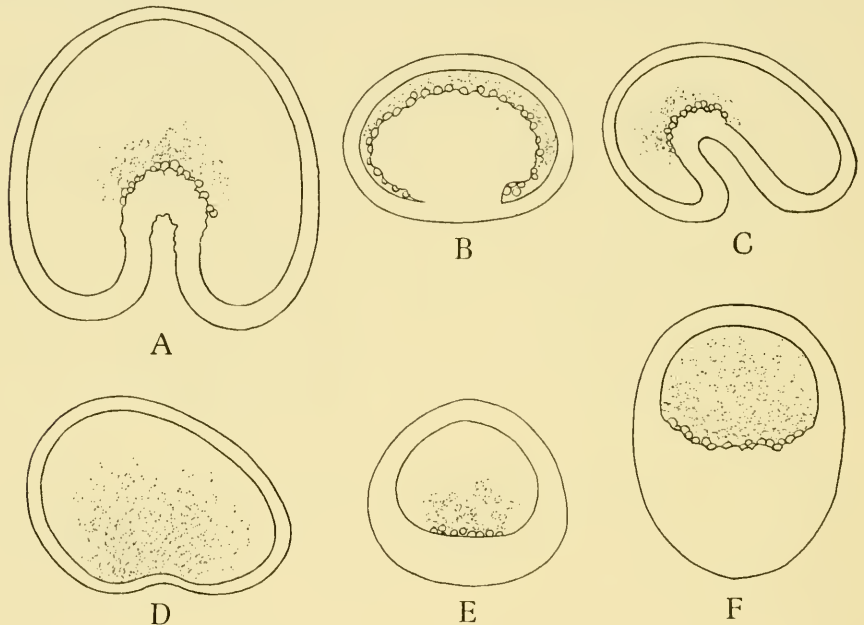


FIG. 85, A-F.—Loss of epithelial character and dissociation of the entodermal region in *Patiria* with relatively extreme differential inhibition continuing to gastrulation or later.

Different degrees of entodermal dissociation with more extreme inhibitions are shown in Figure 85, C-F; in C there is some invagination, and dissociation is beginning apically; in D invagination apparently began, but the whole prospective entoderm, except for a thin external layer, has lost its coherence; in E and F gastrulation is completely inhibited, and the cell immigration from the entodermal region resembles immigration of mesenchyme in echinoids; there is apparently some entodermization in E and in F. In Figure 85, A and B are effects of LiCl, C is from a lot in KCN, D from sea water at pH 7, E from another lot in KCN, and F from

a crowded lot; loss of epithelial character and dissociation of entoderm are evidently not specific effects of a particular agent: they depend on concentration and exposure period. The apparent asymmetry of *C* and *D* results from ventrodorsal differential inhibition. The inhibiting conditions may completely prevent development of ventrodorsality (Fig. 85, *E*, *F*); but if it develops, inhibition decreases from the ventral region dorsally. That it is actually the ventral region that is more inhibited cannot be determined from forms like *C* and *D*, but many individuals undergo sufficient development to permit certain identification of ventral and dorsal regions.⁶ Gastrulae such as *B* and *D* of Figure 85 usually show little or no further development; but forms like *A*, *C*, *E*, and *F* may continue

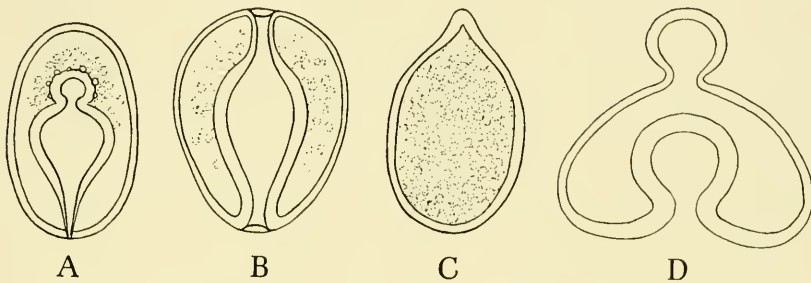


FIG. 86, *A-D*.—Slight secondary modifications in *Patiria* after relatively extreme inhibition. *A*, KCN m/50,000, 18 hr. from two-cell stage, 11 days water; *B*, LiCl m/20, 16 hr. from two-cell stage, 8 days water; *C*, LiCl m/30, 38½ hr. from early cleavage, 8 days water; *D*, LiCl, m/25, 19½ hr. from early cleavage, 5 days water.

development as differentially inhibited forms or show some secondary modification, according to experimental conditions.

The question arises whether the cells dissociating from the entodermal region represent premature mesenchyme, and, if so, how it is possible that inhibiting factors determine their appearance. The data on dye reduction indicate low oxygen in the blastocoel; in the starfish it is so low that the apical region of the archenteron, from which mesenchyme normally dissociates, reduces both methylene blue and Janus green in water of the usual oxygen content and open to the air (Child, 1936*a*). It is suggested that under inhibiting conditions which decrease oxygen or inhibit its intracellular utilization, dissociation may occur without activation of the region concerned or with slight activation. But whether dissociated

⁶ Forms like those of Fig. 85 also appear in low oxygen, in hypotonic sea water, with Janus green, and with ultra-violet radiation.

cells function as mesenchyme or remain as inhibited cells is not known, except for the fact that some of them may form epithelial vesicles in echinoids (pp. 238-39).

SECONDARY AND COELOMIC MODIFICATIONS

Forms resulting from temporary exposure in early stages to severe inhibiting conditions, but with slight secondary modifications, are shown in Figure 86. In *A* the only indication of possible differential recovery is

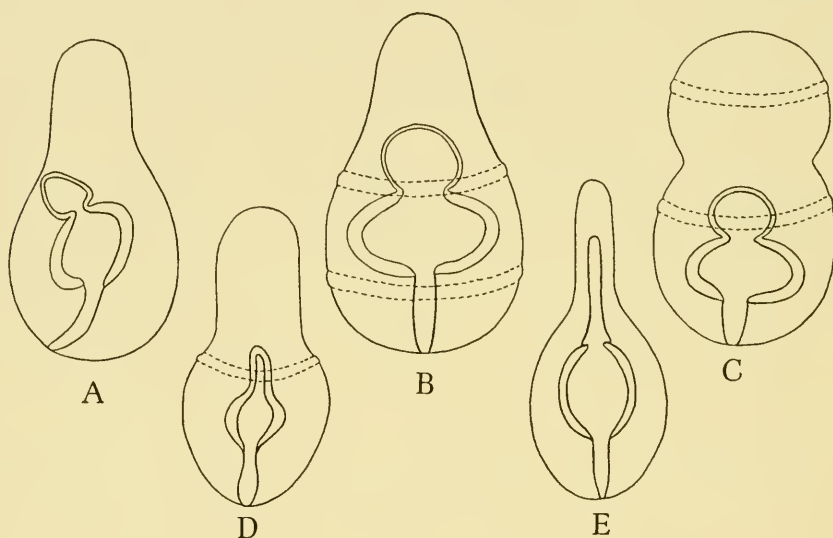


FIG. 87, *A-E*.—Differential recovery in water after differential inhibition. *A, B, Patiria*, LiCl m/20, 10½ hr. from early blastula; *C-E, Asterias*; *C, D*, KCN m/200,000, continuous from one-cell stage but with gradually decreasing concentration after 2 days; *E, Patiria*, LiCl m/30, 21 hr. from two-cell stage.

the elongation, the earlier gastrula being flattened apically; *B* shows similar elongation and an apical mouth; slight apical thickening and outgrowth appear in *C*, but there is complete breakdown of entoderm, except a thin outer layer; apical recovery is evident in *D*. With somewhat less extreme inhibition further development and more or less differential recovery follow return to water, but ventrodorsality may be completely or almost completely obliterated. In Figure 87, *A* and *B*, lithium forms of *Patiria*, have apparently completely radial ectoderms, but the form of the enteron in *A* suggests that some slight ventrodorsality is still present or was present at a stage affecting entodermal development. In *B* there is no evidence of ventrodorsality; and *C, D*, and *E*, modifica-

tions of *Asterias*, are apparently completely radial. In *E* differential recovery involves only the extreme apical region and perhaps the foregut. Similar forms are produced by other agents with certain concentrations and exposure periods; they also appear in eggs from animals kept too long under laboratory conditions. All of them represent some degree of secondary modification, differential tolerance, conditioning, or recovery of a more or less extensive apical region, for they appear only in the lower inhibiting concentrations or after return to water, and they develop from gastrulae with inhibited apical ectoderm.

The development of one or two ciliated bands in some of these forms is of interest (Fig. 87, *B, C, D*). As in many echinoid ectoderms inhibited to radial form, the bands have become transverse circles, that is, their localization is different from the normal. A few individuals with three bands, at least ventrally, have been observed (see Fig. 89, *H*). This localization of ciliated bands suggests approach to the larval pattern of the crinoid with five bands and little development of ventrodorsality in larval form.

Final stages of differentially inhibited forms with ventrodorsality and some degree of apical recovery are shown in Figure 88, *A-D*. In *A, B*, and *C* the whole preoral region is represented only by an ectodermal thickening, in *D* by secondary apical outgrowth; entoderm shows little or no recovery, the foregut being relatively small (*A, B, C*) or not differentiated (*D*). When these forms develop a ciliated band, it is transverse (*A, B*). Special interest attaches to these modifications because they approach in form and proportions the echinoid prepluteus (see Fig. 73, *B, C*). Since the apicobasal gradient of the starfish is apparently steeper than that of the echinoid in earlier stages (Child, 1936a), it is perhaps significant that with decrease of its steepness by differential inhibition there is approach to echinoid form and proportions. These types evidently result from a relatively high degree of differential inhibiting action between apicoventral and other regions. They have been seen most frequently with LiCl, which evidently has a high differential action, but they also appear with other agents.

With somewhat greater degree of differential recovery forms like *E* and *F* of Figure 88 appear, often in the same lots as those of Figure 88, *A-D*. In a given lot animals swimming free or at the surface show, in general, greater recovery than those at the bottom or in aggregations. When the primary inhibition is slight, forms resulting from differential recovery are of the general type of Figure 88, *G*. The preoral region is enlarged; mouth

and foregut are usually relatively large; and the ventral surface is almost or quite flat, rather than concave. All degrees of this modification occur

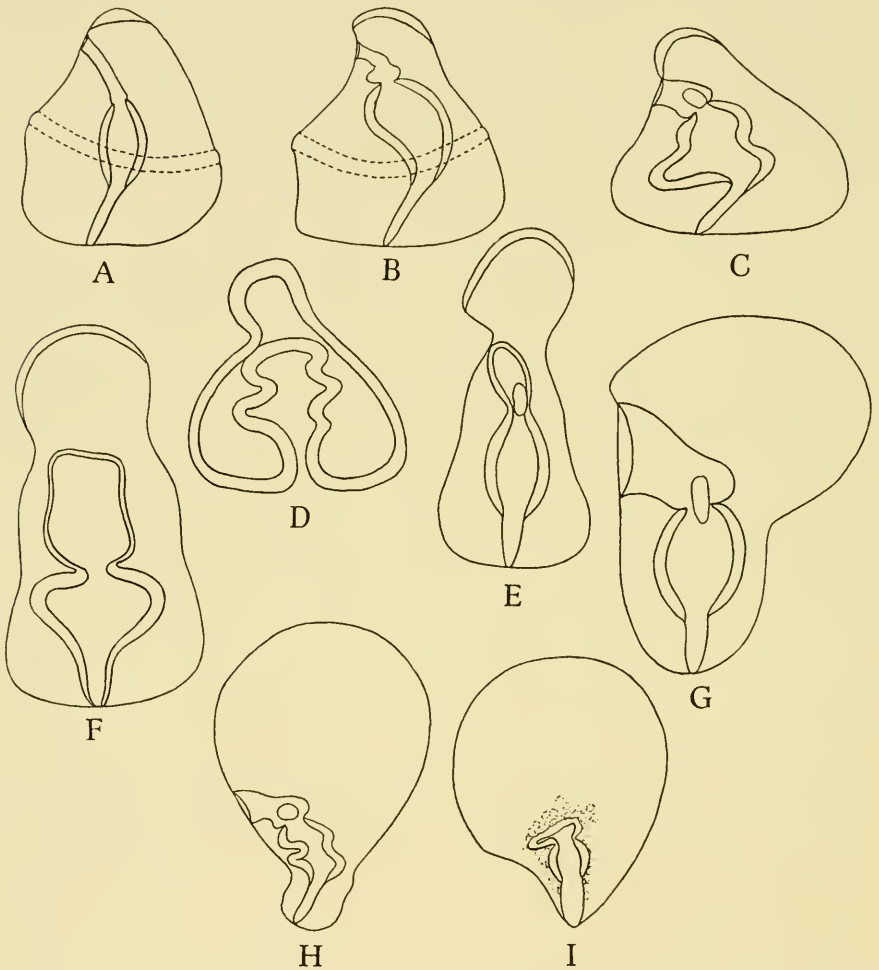


FIG. 88, *A-I*.—Secondary modifications of *Patiria* following differential inhibition. *A-D*, forms with slight apical recovery resembling echinoid preplutei in form, developing in water after LiCl m/20, m/25, m/30, 10–21 hr. from early cleavage; *E*, differential recovery after 21 hr. LiCl m/30 from two-cell stage; *F*, differential recovery after 14½ hr. LiCl m/30 from sixty-four cell stage; *G*, differential recovery after 21 hr. in 80 per cent sea water from two-cell stage; *H*, differential conditioning in 90 per cent sea water 13 days; *I*, secondary modification in Nile blue sulphate, initial concentration 1/1,000,000.

in recovery after exposure to all inhibiting agents used, when inhibiting action is not too great. The modifications resulting from differential tolerance or conditioning with continuous exposure to low ranges of inhibit-

ing action are, in general, similar in type to the differential recoveries but are usually, as might be expected, less extreme and with a degree of secondary modification differing with different agents. For example, secondary modifications are slight in cyanide; but in various other agents, even in LiCl, they may be considerable or even extreme. A characteristic form developing in 90 per cent sea water is shown in Figure 88, *H*; the preoral region is relatively very large and almost spherical, the postoral region and entoderm are still small. Forms of this type develop from gastrulae with slight apical inhibition. In low concentrations of neutral red and Nile blue sulphate similar forms develop. Figure 88, *I*, is an example from a Nile blue sulphate lot in which the dye gradually disappeared, both from the solution (open to the air) and from the animals. Evidently there is a differential in ability to dispose of the dye in some way. Entoderm stains more deeply than ectoderm and may remain differentially inhibited, as in the figure, or show some secondary modification apically.

Of the two starfish coeloms, originating as localized budlike outgrowths on right and left sides of the foregut and separating from it as small vesicles, the left is usually the larger, or becomes larger, gives rise to the madreporic canal, and becomes the hydrocoel. Reversal or obliteration of this asymmetry, as an occasional occurrence under natural conditions, has been noted repeatedly and induced experimentally; and single coeloms, either dorsal or ventral, have also been observed in sea urchins under experimental conditions.⁷ These modifications of coelom development occur in *Patiria* under all inhibiting conditions thus far used by the writer. They are undoubtedly to be regarded as cases of differential inhibition. When the enlarged apical region of the archenteron extends ventrally toward the stomodeal region, growth in length is much greater on the apicodorsal side than on the other. This is evident in the form of the foregut in later stages (Fig. 83, *A*). With differential inhibition of this development the coeloms tend to appear nearer the median line on the dorsal side. All degrees of approximation to the median region appear from a slender connection between the two (Fig. 89, *A*) to development of a single median sac, bilaterally symmetrical with two madreporic canals (Fig. 89, *B*) or with a single median dorsal canal (Fig. 89, *C*). With continuous exposure to inhibiting conditions the differential elongation of the foregut may be completely inhibited so that it remains a blind, rounded, or somewhat elongated region at the apical end of the archenteron. If coelomic development occurs in such cases, it is usually apical

⁷ Newman, 1925, *Patiria*, low temperature; see also Newman, 1921*a*, *b*, 1922; Runnström, 1925*b*, *c*, *Psammechinus*, hypotonic sea water.

and may show some bilaterality (Fig. 89, *E*) or be single and median (Fig. 89, *F*). In some cases when the foregut elongates, a single coelom develops on the ventral side (Fig. 89, *G, H*), between it and the stomodeal region. The various degrees of differential modification from bilateral to median origin of the coeloms with approximation, partial fusion, or single median coelom with bilaterality obliterated present a series of differential

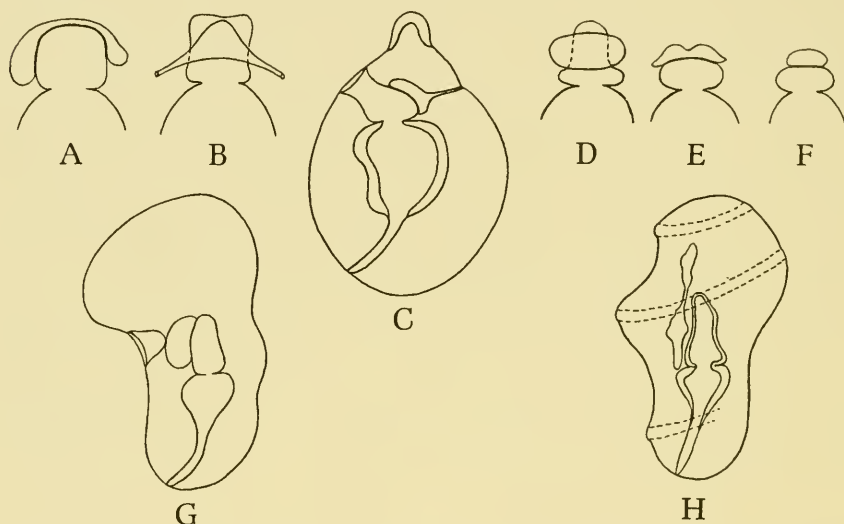


FIG. 89, *A-H*.—Modifications of coelom development by differential inhibition in *Patiria*; outline of foregut and part of midgut with coelom: *A, B, D-F*, from dorsal side, *C, G, H*, from left side. *A*, right and left connected, asymmetry evident; *B*, single symmetrical and median with two canals, both 11 hr. LiCl m/25 from thirty-two cells; *C*, single median dorsal with median dorsal canal, LiCl m/40, continuous from two-cell stage; *D*, single symmetrical without canal, LiCl m/30, 21¼ hr. from two-cell stage; *E, F*, apical (anterior), LiCl m/40, continuous from two-cell stage; *G*, single median ventral, between foregut and stomodeum, eggs from animals long time in laboratory; *H*, single median ventral, LiCl m/30, 38½ hr. from sixty-four cells, undoubtedly some recovery but occurring late, after ventrodorsality of foregut apparently reversed.

inhibitions similar to those of the planarian head, the anal arms of the sea-urchin larva, and the approximation to the median line of eyes and other bilateral organs of the vertebrate head. In all these cases the modifications evidently depend on a mediolateral physiological differential, a median region being more inhibited than lateral; and as inhibition increases, lateral organs appear successively nearer the median line and become median. Coelom development on the ventral side of the foregut probably results from reversal of the ventrodorsal differential by inhibi-

tion, that is, the apicodorsal region is so strongly inhibited that only on the less inhibited ventral side is coelom development possible. Obliteration and reversal of the normal coelomic asymmetry also represent differential modifications and are characteristic results of certain degrees of inhibition by all agents used.

It is possible that with sufficient inhibition of the more susceptible region the normally less susceptible and presumably less active region is, to some extent, physiologically isolated and undergoes activation, particularly after return to water. If the left coelom normally represents a higher level of activity and is in some degree dominant, differential inhibition may permit sufficient activation of the right side to reverse asymmetry. Dorsiventral differential inhibition of the foregut may bring about physiological isolation and permit activation of the ventral side and development of a coelom there after return to water. But whether physiological isolation is or is not involved, it seems evident that localization of coelom development on the foregut is determined by nonspecific factors, quantitative differences in physiological condition, rather than by definitely localized, "coelom-forming substances." With recovery after relatively extreme inhibition of the apical entodermal region three or even four coelom buds may develop, usually equidistant from each other, about the circumference of the radial or almost radial foregut. In these cases asymmetry has been obliterated, there is little or no evidence of ventrodorsality, and the whole circumference of the foregut is apparently equipotent for coelom. The question how the coelom buds are localized about the circumference of the foregut in a particular case is the same as that concerning localization of individual tentacles about the circumference of the body in various hydroids. The small coelomic vesicles developing from the posterior region of the midgut also show change in position, number, and asymmetry under inhibiting conditions and with secondary modifications. In recoveries as many as six of them may develop in a circle about the midgut.

ECHINODERM EXOGASTRULATION AND ASSOCIATED MODIFICATIONS

It was discovered by Herbst (1892, 1895, 1896a) that when early stages of sea-urchin development were treated for a time, beginning soon after fertilization or in early cleavage, with lithium (usually LiCl) in certain concentrations the entodermal region evaginated at the stage of gastrulation instead of invaginating. Another effect of lithium was differential entodermization of prospective ectoderm, progressing from basal ecto-

dermal levels acropetally with increase in lithium effect, until in extreme cases all the ectoderm had become entoderm and an organism consisting only of entoderm and mesenchyme resulted. All gradations from the normal gastrula to these completely entodermized forms were found, according to conditions; forms with partly evaginated, partly invaginated entoderm, entexogastrulae or exentogastrulae, were also described. Herbst was at first inclined to regard exogastrulation as a more or less specific effect of lithium; but he soon found that sodium butyrate and lack of magnesium would also produce exogastrulation (Herbst, 1895, 1897). Since Herbst's work exogastrulation has been produced by many investigators in various species of sea urchins and several species of starfish not only by lithium but by a great number of other agents, both chemical and physical.⁸ Probably further experiment will add other agents to this list. In the light of the data at hand it is evident that exogastrulation is not a developmental modification determined by specific action dependent on the chemical composition or specific physical effect of a particular agent or agents and specific regional differences in egg or embryo. It is extremely improbable that all agents listed in the footnote act in the same manner on particular regions of sea urchin, sand dollar, and starfish embryos; but if they do not, the question at once arises whether exogastrulation may not be, like other modifications described in preceding sections, a differential modification, depending on nonspecific alterations of a primarily quantitative gradient pattern.⁹ A recent study of differential dye

⁸ E.g., exogastrulation has been produced by NaCl, HgCl₂, CuSO₄, KCN, NiCl₂, and by various other salts and salt mixtures (MacArthur, 1924; Waterman, 1932, 1934, 1938), by crowding and acidified sea water (MacArthur, 1924; Child), by high and low temperatures (Driesch, 1893; Waterman, 1934), by neutral red (MacArthur, 1924), by methylene blue and Janus green (Child), by auxin, glycogen, and HClO₃ (Motomura, 1934), by tobacco smoke (Child), by lack of oxygen (Child), by X-rays, and occasionally a few exogastrulae by respiratory stimulants (Waterman, 1934, 1938). It has been observed by Runnström (1933) that lithium and CO are additive in effect. In crowding lack of oxygen is probably the effective factor rather than CO₂, for the modifications occur when pH has not decreased below 7.3 or 7.4; but it is possible that an excreted toxic metabolite may be concerned. In acidified sea water CO₂ is probably the inhibiting factor.

⁹ The writer's interest in the problem of exogastrulation, extending over many years, has led, as opportunity offered, to a wide range of experiment, particularly with LiCl in concentrations ranging from the lowest effective to those completely inhibiting development and killing rapidly and with exposure periods beginning at various developmental stages and ranging from an hour or two to continuous, also some exposures to inhibiting agents before fertilization. The following species have been used as material: echinoids: *Arbacia punctulata*, *Strongylocentrotus franciscanus*, *S. purpuratus*, *Echinarachnius parma*, and *Dendraster excentricus*; asteroids: *Asterias forbesii* and *Patiria miniata*. For the most recent general discussion of echinoderm exogastrulation, a paper not included in the Bibliography, see Child, 1940, "Lithium and echinoderm exogastrulation," *Physiol. Zool.*, 13.

reduction in relation to exogastrulation suggested an interpretation of this series of developmental modifications, differing somewhat from hypotheses advanced by others (Child, 1936b). Further experiment, chiefly with *Dendraster* as material, appears to throw some additional light on conditions concerned in development of these extremely interesting forms.

ECHINOID EXOGASTRULAE: A FEW EXAMPLES OF FINAL STAGES

Developmental modifications of exogastrulae are essentially similar in the echinoid species thus far used as material. The following account is chiefly concerned with lithium exogastrulae of *Dendraster excentricus*. Figure 73, *A-E*, showing forms of gastrula and later stages under natural conditions, will serve for comparison with exogastrulae. A few of the almost innumerable modifications of form and proportion in exogastrulae are outlined in Figures 90 and 91,¹⁰ most of them lithium forms developing with different concentrations and exposure periods, beginning at, or soon after, the first cleavage. Attention is called first to the differential inhibitions of the ectoderm in these forms. With the lower concentrations or relatively short exposures ectoderm may attain more or less completely pluteus form (Fig. 90, *A, B*). Figure 90, *C*, a result of crowding, shows somewhat less advanced ectodermal development. With greater inhibition ectoderm is smaller and oral lobe is almost completely inhibited, but arms may develop at varying wide angles up to 180° (Fig. 90, *D-F*). As will appear more clearly below, the basal part of the prospective ectoderm of these forms has been entodermized by lithium, that is, has become entoderm; consequently, the arms develop from ectoderm much farther apical than the original arm area. These forms are similar as regards ectodermal development to the inhibited, wide-angled modifications discussed above (pp. 200-201) and undoubtedly result from essentially similar conditions. They appear in a wide range of concentrations according to exposure period. More inhibited ectoderms develop parallel, fused, or single arms with double- or single-arm skeleton, and in some of them a stomodeum develops in a position relative to the arm, which indicates that the arm is ventral and median (Fig. 91, *A*), as in the one-armed forms

¹⁰ These and following figures concerned with exogastrulation in *Dendraster* are drawn to the same scale from ocular-micrometer measurements of living individuals and sketches of the same individuals made at the same time. Levels of localization of mesenchyme are indicated; but, as regards actual numbers of cells of mesenchyme or dissociated entoderm in the blastocoel, the figures are diagrammatic, though they attempt to show whether free cells are numerous or few and to represent loss of epithelial character and dissociation in entoderm. Pigment cells are indicated, chiefly along the ciliated band, where they are most numerous when it develops. *Patiria* exogastrulae in Fig. 95 are on a smaller scale.

of Figure 74. With further increase of inhibiting action the ectoderm is radial; the ciliated band, if it develops, is localized around the basal region (Fig. 91, *B-D*); and if skeleton develops at all, it forms an irregular ring of spicules, often subapical (*B*) instead of basal. In absence of skeletal development a ring of mesenchyme may occupy the same position (*C, D*).

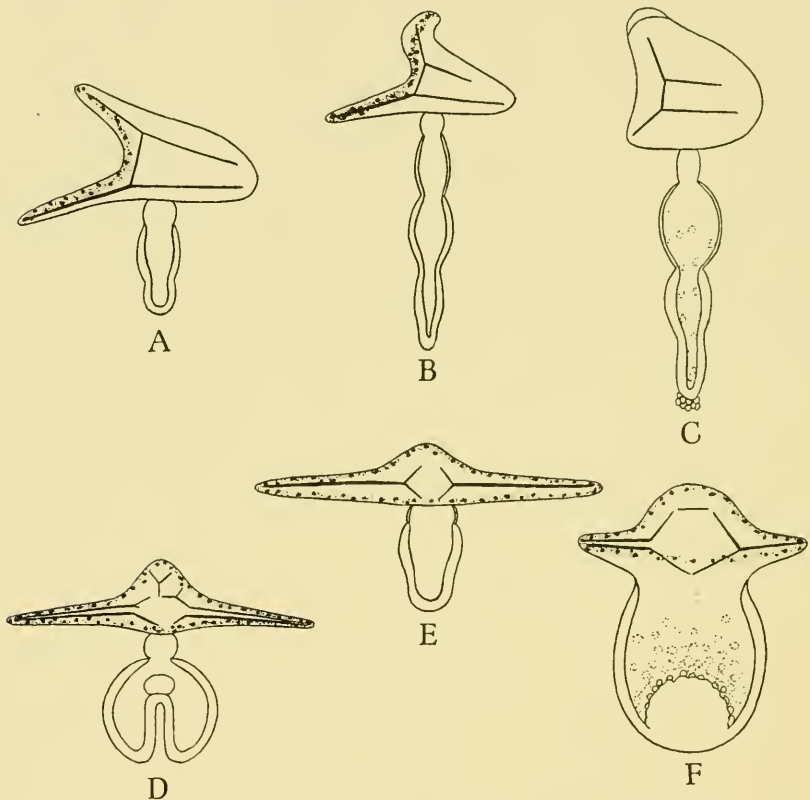


FIG. 90, *A-F*.—Less extreme types of *Dendraster exogastrulae*. *A*, LiCl m/90, continuous from early blastula; *B*, LiCl m/40, 14 hr. from early blastula; *C*, crowded 10 hr. from early blastula; *D*, LiCl m/25, 5 hr. from first cleavage; *E*, m/70, continuous from first cleavage; *F*, LiCl m/60, continuous from first cleavage.

Further reduction of ectoderm is shown in Figure 91, *E-G*. Position of the skeletal rods in *E* is probably not indicative of the original ventro-dorsality, and form of the ectoderm appears to be determined by their elongation. In *F* and *G* their direction is apicobasal; occasionally in such forms a short armlike outgrowth develops at the apical pole, apparently induced by the skeleton. In Figure 91, *H*, the only part of the original

ectoderm remaining is the tiny button at the apical pole. A thin-walled "neck" connecting external entoderm and ectoderm develops in many exogastrulae, apparently always as a secondary modification, not as a direct effect of lithium (Figs. 90, *A-E*, and 91, *A-C, E-G*), and apparently of ectodermal origin. Its possible significance is discussed later.

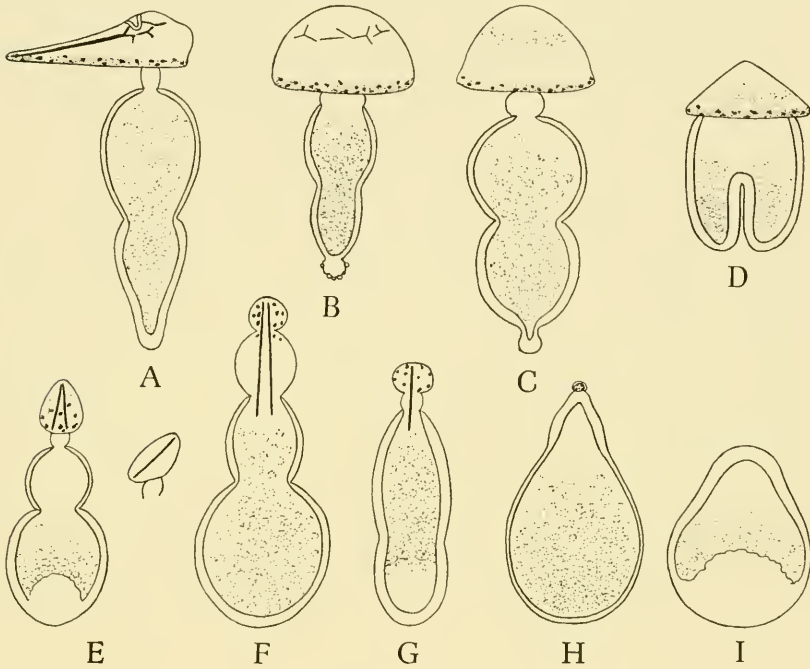


FIG. 91, *A-I*.—More extreme types of *Dendraster* exogastrulae: final stages attained in water after various exposures to LiCl. *A*, *m/25*, 7 hr. from first cleavage; *B*, *m/30*, 18½ hr. from eight cells; *C*, *m/25*, 7 hr. from first cleavage; *D*, *m/50*, continuous from first cleavage; *E*, *m/30*, 18½ hr. from first cleavage; *F*, *m/25*, 7 hr. from first cleavage; *G*, *m/20*, 9 hr. from early blastula; *H*, *I*, *m/20*, 9½ hr. from four cells.

Exogastrular entoderm also shows a wide range of modifications. The original entoderm may invaginate more or less but is inhibited in further development (Figs. 90, *D*, and 91, *D*); or, with more extreme inhibition, it may lose epithelial character and dissociate more or less completely and the exogastrular entoderm develops in large part, perhaps in some cases entirely, from entodermized ectoderm (Figs. 90, *F*, and 91, *E-H*). It is often impossible to determine how large a part of the total entoderm is original prospective entoderm. On the other hand, with relatively slight

inhibitions, all or almost all of the exogastrular entoderm may develop from the evaginated original entoderm (Fig. 90, A-C). With the less extreme inhibitions three entodermal regions may be more or less distinctly marked off by constrictions (Fig. 90, B, C). In recovery after greater in-

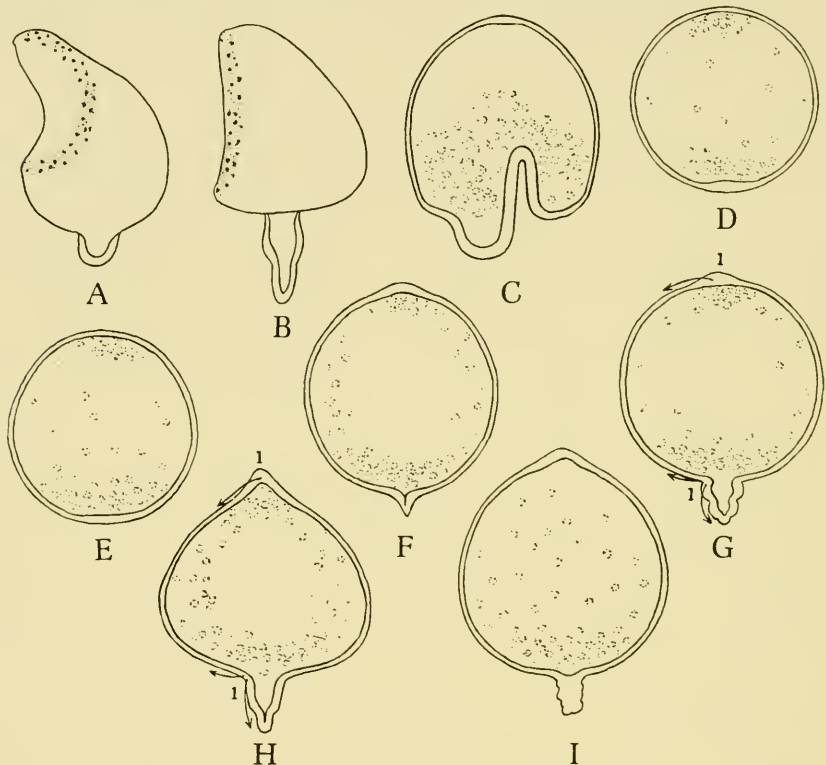


FIG. 92, A-I.—Echinoid exogastrulae with small entoderms, resulting from exposure to LiCl beginning in later pregastrular stages or at beginning gastrulation. A-C, *Dendraster*; D-G, *Strongylocentrotus purpuratus*; H, I, *S. franciscanus*. A, B, m/45, 39 hr. from 6-hr. blastula; C, m/25, 24 hr. from beginning gastrulation; D-G, m/40, 20 hr. from late blastula; H, I, m/40, 24 hr. or more from late blastula. Arrows in G and H indicate directions of progress of dye reduction in low oxygen and numerals 1 and 1, that dye reduction becomes evident at about the same time in the regions indicated (D-I from Child, 1936b).

hibition development of two entodermal regions of large size is common, often with a third region of small size or barely indicated (Fig. 91, A-C, E-G). After extreme inhibition there is little or no regional differentiation (Fig. 91, H).

When exposure to lithium begins in late pregastrular stages or, in moderate concentration, continues up to, or through, gastrulation, entoderm

is generally more inhibited than ectoderm; and if exogastrulation occurs, the entoderm is usually relatively small (Fig. 92, *A-C*). Figure 92, *C*, is an entexogastrula in which partial invagination preceded evagination, but entoderm is evidently inhibited in development. Forms resulting from exposure of *Strongylocentrotus* to lithium in late pregastrular stages are shown in Figure 92, *D-I*. It was suggested in an earlier paper (Child, 1936*b*) that in cases like *D-F* of Figure 92 more or less ectodermization of prospective entoderm has taken place. Cells in the blastocoel do not indicate extensive dissociation of entoderm.

DEVELOPMENT OF ECHINOID EXOGASTRULAE

The final forms of many exogastrulae give little information concerning their developmental physiology: for such information it is necessary to look to the earlier stages, as modified by different concentrations and exposure periods. Since the pioneer work of Herbst it has been known that lithium retards and, in sufficient concentration, completely stops development and kills. That entodermization of prospective ectoderm may occur in connection with exogastrulation was also observed by Herbst. Lithium is believed by Runnström and his co-workers to have a specific action on the postulated vegetative concentration gradient. In recent papers it is maintained that lithium inhibits the animal kind of metabolism and increases the vegetative kind.¹¹ We have to inquire whether, or to what extent, the development of exogastrulae supports this hypothesis.

Normally the primary mesenchyme after immigration and before bilateral aggregation is localized in a more or less definite ring or circle on the inner surface of the ectoderm near its junction with the prospective entoderm. As is well known, this ring may be localized much farther apically in developing exogastrulae; that is, the ectodermal level which localizes it is displaced apically. This shift apically of certain unknown conditions in the ectoderm is usually associated with entodermization of the more basal levels of prospective ectoderm, though slight apical shift is apparently possible without appreciable entodermization. Lithium has still another effect: it inhibits development of entoderm. That it entodermizes ectoderm and also inhibits entoderm is not the paradox that it may appear to be, but is very simply interpreted. And, finally, there is evidence indicating that some ectodermization of prospective entoderm may occur under certain conditions, particularly in recovery after return

¹¹ See, e.g., Lindahl, 1936, p. 339, etc., and references to earlier papers by Runnström; also Runnström, 1928*b*, 1935*a*.

to water. The maximum inhibiting and modifying effect of a given concentration of LiCl is attained only gradually, presumably according to rate of penetration. High concentrations are more rapidly effective than low; and the effects of the higher concentrations, even with short exposure, are more persistent than those of low, perhaps because intracellular concentration remains above the threshold of effectiveness for a longer time. Within certain limits longer exposure to the lower effective concentrations

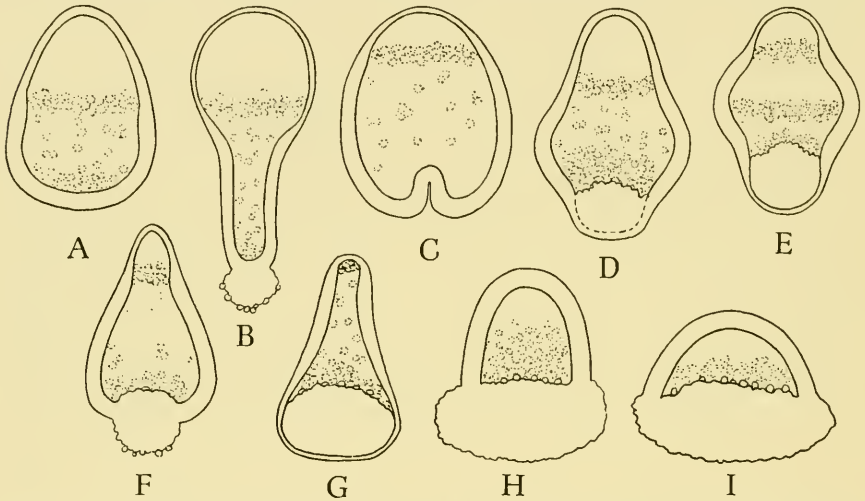


FIG. 93, A-I.—Developmental stages of *Dendroaster exogastrulae* showing displacement apically of primary mesenchyme (A-G), entodermization of prospective ectoderm (A-I), and loss of epithelial character and dissociation of prospective entoderm (B, D-F) and of basal parts of entodermized ectoderm (G-I). A, LiCl m/50, 14½ hr. from first cleavage, identical forms in crowded lots; B, crowded 10 hr. from early blastula, 6 hr. water; C, LiCl m/30, 5 hr. from first cleavage, 18 hr. water; D, E, LiCl m/60, 25 hr. from first cleavage; F, LiCl m/25, 7 hr. from first cleavage, 14 hr. water; G, LiCl m/20, 9 hr. from first cleavage, 18 hr. water; H, LiCl m/30, 18½ hr. from first cleavage; I, crowded 27 hr. from first cleavage.

gives modifications essentially similar to those resulting from shorter exposure to higher concentrations.

Early stages of exogastrulae with different degrees of entodermization and displacement apically of mesenchyme are shown in Figure 93. In A of this figure, a rather long exposure to a relatively low concentration, the apical region is obviously inhibited, and almost the whole basal half of the ectoderm has become entoderm. With continued exposure the original entoderm may invaginate more or less but is inhibited in further development, the entodermized ectoderm becomes the external entoderm of the exogastrula, and ventrodorsality is usually obliterated in the remaining

ectoderm (Fig. 91, *D*). This figure represents the most advanced stage attained with continuous exposure to LiCl m/50. Evidently both invaginated entoderm and entodermized ectoderm are inhibited in development, and some dissociation of entoderm has occurred. After return to water at the stage of Figure 93, *A*, ectoderm may develop more or less ventrodorsality (Fig. 90, *D*, *E*), and entoderm may show some further development. Figure 93, *B*, shows entodermization resulting from crowding in early stages; after return to water, forms of the general type of Figures 90, *C*, result. Evidently, entodermization is not a specific effect of lithium. Figure 93, *C*, a short exposure to rather high lithium concentration, shows extreme entodermization and greatly inhibited invagination, even after 18 hours in water. With further recovery, however, ectodermal ventrodorsality appears in many individuals, the most advanced attaining approximately the condition of Figure 90, *D*, with all gradations from this form to completely radial forms like Figure 91, *D*. In all, the later development of original entoderm and entodermized ectoderm remains inhibited.

With higher concentrations or longer exposures the original entoderm may lose, in part or wholly, its epithelial character and become a solid cell mass from which cells dissociate internally or externally or both, though in some individuals a thin external layer remains epithelial or regains epithelial character after return to water (Fig. 93, *D-G*). Crowding has a similar effect (Fig. 93, *B*). With still more extreme inhibiting conditions, either by long exposure or by high concentrations, loss of epithelial character and dissociation progress acropetally in the entodermized ectoderm (Fig. 93, *H*, *I*). In the most advanced stages attained in recovery from these effects more or less of the original entoderm may remain a cell mass, internal or external, at the tip of the entodermized ectoderm, or an external layer may constitute epithelium at the free end of the exogastrular entoderm.¹²

In these forms it is impossible to determine how much of the exogastrular entoderm develops from the original prospective entoderm; but the earlier stages, such as *E-G* of Figure 93, indicate that the external entoderm may consist mostly, perhaps in some cases wholly, of entodermized ectoderm. Dissociated entodermal cells do not usually appear to take any part in further development, though it is uncertain whether they may function as mesenchyme in some cases of recovery. Usually, however, with inhibition sufficient to produce entodermal dissociation, little or no skeleton develops.

¹² See Fig. 91, *B*, *C*, *E*, *G*, *H*, *I*, and probably also *F*; also Fig. 93, *D*, *E*, *G*.

With these more extreme lithium effects mesenchyme is localized near, or even at, the apical pole, indicating entodermization of most of the ectoderm (Fig. 93, *E-G*); with complete entodermization it may not be definitely localized (Fig. 93, *H, I*). Occasionally two circles of mesenchyme appear at different levels (Fig. 93, *E*), and in a crowded lot individuals with three circles have been observed.

With concentrations of LiCl high enough to stop development in rather early stages, particularly with sea-water solutions, which are hypertonic, entodermization of ectoderm is apparently complete, and the basal region becomes extremely thick, with partial or progressive obliteration of the blastocoel (Fig. 94). Under these conditions many individuals become spherical solid cell masses. With return to water before lethal effect, forms

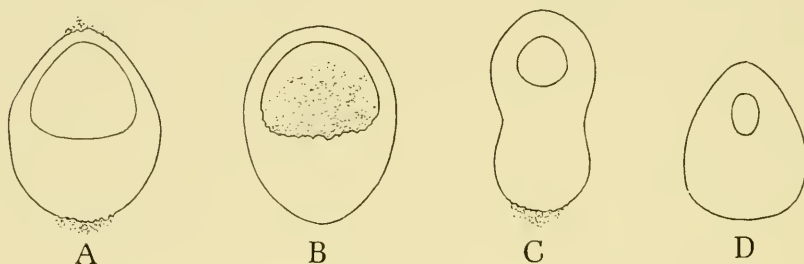


FIG. 94, *A-D*.—Entodermization with continuous exposure to high concentrations of LiCl from early cleavage. *A, B*, m/25, 26 hr.; *C, D*, m/20 in sea water (hypertonic).

like *A* and *B* of Figure 94 may become exogastrulae like Figure 91, *E-H*, even though they appear earlier to be completely entodermized.¹³

Exogastrulae of *Strongylocentrotus*, *Arbacia*, and *Echinarachnius*, and, so far as can be determined from the work of others, those of other echinoid species, do not differ essentially from *Dendraster* exogastrulae. Differences in proportions of certain parts, particularly in the secondary modifications, apparently result from differences in steepness of the gradients and in degree of activation of entoderm in different species; but as regards the general types of modification, there is a high degree of similarity.

ASTEROID EXOGASTRULATION

General characteristics of asteroid and echinoid exogastrulation are similar, but there are minor differences resulting from absence in the starfish of mesenchyme formation preceding gastrulation and from the later

¹³ In the hypertonic solutions (Fig. 94, *C, D*) there is little or no dissociation, probably because of hypertonicity; but after return to water it may be extensive or more or less complete.

increase in susceptibility and apparent activation of entoderm (pp. 134-39). Exogastrulae of the starfish *Patiria* (Fig. 95) show various de-

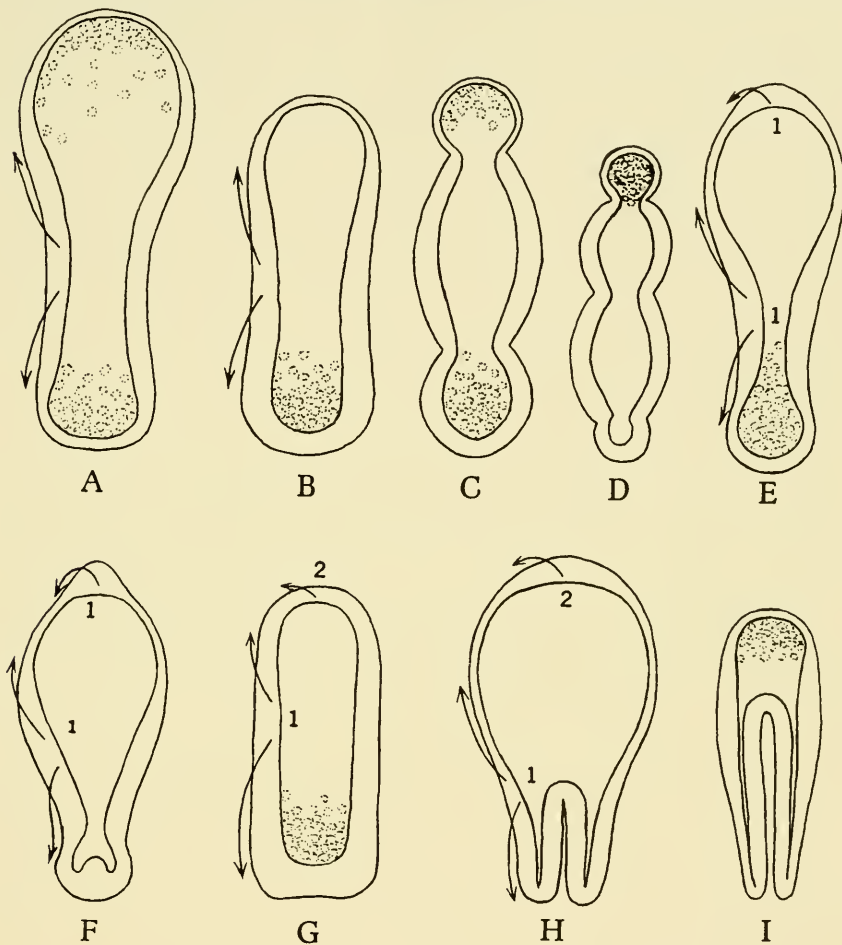


FIG. 95. *A-I*.—Lithium exogastrulae of *Patiria*. *A-F*, continuous exposure from two-cell stage: *A*, m/30, 60 hr.; *B*, m/40, 77 hr.; *C*, *D*, m/40, 95 hr.; *E*, *F*, m/40, 94 hr., slight secondary modification apically. *G-I*, differential recoveries: *G*, *H*, m/30, 18 hr. from first cleavage, 2 days water; *I*, m/20, 19 hr. from first cleavage, 3 days water; *H* and *I*, exentogastreae. Arrows indicate directions of progress of dye reduction in low oxygen, and numerals 1 and 2 the time-order in which reduction becomes evident in different regions (from Child, 1936b).

gresses of entodermization of prospective ectoderm; cells often dissociate from entoderm into the blastocoel, resembling the primary mesenchyme of echinoids (Fig. 95, *A-C*, *E*, *G*); and the apical ectoderm, when strongly

inhibited, also gives off cells (Fig. 95, *A, C, D, I*). Thus far, little differentiation has been seen in the remaining ectoderm of starfish exogastrulae, even in recovery. In forms with considerable entodermization the remaining ectoderm represents more or less of the prospective preoral region, but there is no evidence that it reconstitutes a more or less complete larval ectoderm. It may become rounded and in some individuals an apical thickening appears as a secondary modification (Fig. 95, *E, F*). Extensive, perhaps complete, entodermization of ectoderm and loss of epithelial character in the prospective entoderm results in forms like Figure 93, *G*, of *Dendraster*, an effect of long-continued exposure in both; and more or less entodermal dissociation may occur in later gastrula stages without entodermization or exogastrulation (see Fig. 85). Whether dissociated cells of the prospective entoderm may function as mesenchyme is not known; but here, as in echinoids, they appear to be merely inhibited cells without particular function.

In the asteroid, as in echinoids, lithium entodermizes prospective ectoderm and, after the increase in entodermal susceptibility occurs, also inhibits entoderm. Exogastrulation has also been produced in *Patiria* by crowding and by acidified sea water, by KCN, and by Janus green; in *Asterias* by lack of oxygen. With all these agents there is apparently more or less entodermization of prospective ectoderm. Probably still other agents will produce exogastrulation and entodermization in starfish, as in echinoids.

EXOGASTRULAE AND PSEUDO-EXOGASTRULAE

In this discussion of exogastrulation all forms with external entoderm have been called "exogastrulae," but they are not all alike in origin. Strictly speaking, exogastrulae are modifications in which the entoderm evaginates instead of invaginating. In many so-called "exogastrulae," however, the original prospective entoderm invaginates, and the external entoderm is entodermized ectoderm (Figs. 90, *D*, and 91, *D*). These have been called "entexogastrulae," or, when the invagination is secondary, "exentogastrulae" (Fig. 95, *H, I*). In many cases, also, the prospective entoderm takes little, perhaps no part, in formation of the external entoderm but loses epithelial character and remains a cell mass, usually with more or less complete dissociation, while the external entodermal epithelium is, in large part or wholly, entodermized ectoderm, as in Figures 91, *B, C, E, G*, and *I*, and probably also in *F* and *H*. The entodermized ectoderm of these forms has not evaginated instead of invaginating, and it shows no tendency to invaginate after return to water. If evagination re-

sults from a reversal of cell polarity in the prospective entoderm, there is no evidence of such reversal in entodermized ectoderm. It remains in essentially the same physical relations to other parts as when it was prospective ectoderm of the blastula. So-called "exogastrulae" of this type are actually not exogastrulae at all but partly entodermized blastulae with more or less regional differentiation. For convenience and in a purely descriptive sense they may be called "exogastrulae" because their entoderms are external instead of internal, but it should not be forgotten that, as regards origin and development, they are very different from true exogastrulae; in so far as the entodermized ectoderm is concerned, they are pseudo-exogastrulae. Entodermization of ectoderm apparently has no relation to gastrulation.

Since the physical relations of entodermized ectoderm to other parts are not altered by its entodermization and there is no evidence of reversal of polarity in its cells, it remains a question whether the external entoderm formed by it is inside out and which end is physiologically its apical end. The end attached to the remaining ectoderm has developed from the higher, more nearly apical, level of the prospective ectoderm. Moreover, differential dye reduction indicates that this end usually shows higher rate of dye reduction.¹⁴

EXOGASTRULATION AS A DIFFERENTIAL MODIFICATION OF DEVELOPMENT

In general, the modifications of development by lithium do not differ essentially from those produced by other inhibiting agents. All degrees of inhibition of ectodermal development appear with lithium, as with other agents; and the secondary modifications resulting from differential tolerance, conditioning, or recovery are the same in character with lithium and other agents. Even entodermization of prospective ectoderm is not a specific effect of lithium but is produced by various other inhibiting agents—crowding and Janus green in *Dendraster*, several agents in *Patiria*; further investigation will probably show that many other agents have a similar effect. In fact, the evidence suggests that entodermization may be a nonspecific differential inhibition. Differential susceptibility to gradually lethal action of many agents and to inhibiting action on development and differences in rate of dye reduction in low oxygen¹⁵ indicate a single gradient of physiological condition in earlier pregastrular stages of echinoids and asteroids, with decrease in susceptibility and rate of dye

¹⁴ Fig. 95, *A, B, F, G, H*, and Child, 1936*b*.

¹⁵ Chap. iv and preceding sections of present chapter.

reduction basipetally from the apical pole and a change in condition, apparently an activation, in the basal region—in echinoids as gastrulation approaches, in asteroids somewhat later. Prospective entoderm constitutes the lower levels of the primary gradient. Exposure to lithium and other agents entodermizes only when it begins in the earlier pregastrular stages. In view of all the facts, it seems probable that the primary action in entodermization is a depression or inhibition, rather than a direct increase, in concentration and extent of a specific vegetal substance gradient or stimulation of a specific vegetal metabolism, as Runnström maintains. Lower levels of prospective ectoderm are entodermized by lesser degrees of inhibition than higher levels because they are nearer the critical level. This depression must result in alteration of concentration of many reacting substances in the cells concerned and in their relations to other parts, and so in progressive increase of their specificity as entoderm. It has been pointed out that lithium not only entodermizes but inhibits development of original entoderm and of entodermized ectoderm. It is a point of some importance that the exogastrulae with extremely large entoderms (Fig. 91, *B, C, E-G*) are not direct effects of lithium or other agents but secondary modifications, appearing in most extreme form in recovery but also to some extent with differential tolerance or conditioning in low concentrations. Comparison of Figure 90, *A, E*, and *F*, continuous exposures, with Figure 90, *B* and *C*, recoveries after return to water, and of Figure 91, *D*, continuous, with Figure 91, *A-C* and *E-G*, recoveries, will show the difference in entodermal development.

In recovery after high concentrations of lithium, which practically stop development in earlier pregastrular stages, secondary modifications are often greater, if exposure is not too long, than after somewhat lower concentrations, apparently because with the higher concentrations, the stage of activation of entoderm is reached only after return to water, while with lower concentrations it may be reached and activation inhibited during exposure. A comparison of Figure 91, *A, C*, and *F*, 7 hours in $\text{LiCl } m/25$ from two-cell stage, with Figure 91, *D*, in $m/50$ from the same stage, illustrates the point.

Lithium, like other agents, inhibits more or less completely skeletal development; but in secondary modifications, particularly in recovery from the less extreme inhibitions, excess of skeleton with supernumerary arms and other skeletal structures not normally present often develops. Whether mesenchyme cells increase in number secondarily or dissociated entodermal cells function as mesenchyme is not known.

The possibility that specific vegetal substance is already present in early stages is, of course, not excluded; but the evidence seems to support the view that the primary effect of lithium in entodermization, as in other modifications, is depression or inhibition, an action on quantitative differences in physiological condition, with origin or increase of specific differences as a result. In short, according to this view, lithium and many other agents are primarily inhibitors of early echinoderm development, with regional differentials in effect depending on nonspecific differences in physiological condition and their changes in the course of development. Secondary differential modifications of tolerance, conditioning, or recovery are not direct effects of the inhibiting agents but results of action of the gradient factors of physiological pattern.

There is considerable evidence that ectodermization of prospective entoderm and re-ectodermization of entodermized ectoderm are possible. The forms of *Strongylocentrotus* in Figure 92, *D*, *E*, and *F*, with exposure to lithium beginning at late blastula stage, suggest partial ectodermization, as does Figure 92, *A*, of *Dendraster*. At the late blastula stage, or a little later, entoderm becomes more susceptible than ectoderm; and it seems not impossible that with depression or inhibition it may still be ectodermized. The ectodermized and "animalized" forms discussed below (pp. 243-45) have a different origin.

In exogastrulae with ectoderm decreased by entodermization the localization of mesenchyme near the apical ectodermal pole suggests that entodermization extended earlier to, or almost to, that level but that with secondary modification more or less re-ectodermization has left the mesenchyme where it was localized by the entodermization (Fig. 91, *A-D*). Localization of mesenchyme at two or more levels may represent stages in entodermization or in re-ectodermization. It was noted above that individuals with apparently completely entodermized ectoderm (Fig. 94, *A*, *B*) may, in recovery, become exogastrulae with some ectoderm, like Figure 91, *E-H*. These cases also indicate re-ectodermization. In a different line of experiment re-ectodermization has been observed by von Ubisch (1925a, 1929).

At present there seems to be no adequate reason for regarding either true or pseudo-exogastrulae as primarily anything but results of differential inhibition, often with various degrees of secondary modification due to differential tolerance, conditioning, or recovery. The question how any agent brings about true exogastrulation remains. What determines evagination instead of invagination? If we knew how invagination is deter-

mined, we might hope to answer this question; but, although various hypotheses have been advanced in terms of mechanical factors, differential growth, specific constitution of the basal region, differential colloidal swelling inside and outside, etc., they remain hypotheses until we know more about living protoplasms and how external agents act on them. The hypothesis of differences in colloidal swelling as the determining factor not only in gastrulation but in other invaginations, and of its reversal by agents producing exogastrulation, was advanced by Spek (1918). Evagination of prospective entoderm suggests reversal of polarity of some sort in the cells of this region. Differential dye reduction indicates lower oxygen content in the blastocoel than outside (see pp. 133-40). Conceivably, this differential or some other between blastocoel and exterior may induce gastrulation, either by colloidal changes or otherwise, and the activation of the prospective entoderm preceding gastrulation in echinoids may be expected to make it more susceptible to such a differential; but in the starfish the activation is apparently less marked before gastrulation than in echinoids. Perhaps the agents which bring about evagination obliterate or reverse a polarity in the entodermal cells. According to Lindahl (1936), lithium inhibits one component of respiration in sea-urchin embryos. A point of some interest is that the stomodeum, instead of invaginating, often evaginates and is considerably enlarged in exogastrulae, suggesting that exogastrulation is not primarily concerned with a specific vegetal gradient.

There is, however, no evidence of such reversal in cells of entodermized ectoderm. They retain their original relations to other parts and to the blastocoel and show no tendency to invaginate, even in the most complete recoveries. Conceivably, either their failure to invaginate may be due to insufficient activation and susceptibility to the surface-interior differential, or in inhibited forms, as all these are, the differential between blastocoel and exterior may be insufficient to bring about reaction or in some cases may be altered in character by the dissociated cells in it. But whatever the physiology of exogastrulation, it is sufficiently evident that neither true exogastrulation, that is, evagination of prospective entoderm, nor pseudoexogastrulation, development of an external entoderm from entodermized ectoderm, are specific effects of lithium.

RECONSTITUTION IN EXOGASTRULATION

Development of exogastrulae with more or less entodermization of prospective ectoderm may involve extensive reconstitution. All ectodermal

differentiations except those of the extreme apical region and perhaps the skeleton develop in other than their original prospective regions or cells. Anal arms, if they appear, develop from various levels, all apical to the prospective levels. If ventrodorsality is not obliterated and a ciliated band develops around the ventral side, all except its apical levels develop from other cells than normally. When ventrodorsality is obliterated and the band develops around the basal ectoderm (Fig. 91, *B, C, D*), its localization is, in large part, different from normal. The scale of ectodermal organization may be greatly decreased, so that the apical half or even less of the prospective ectoderm may approach pluteus form. This decrease in scale is similar to that occurring in hydroid reconstitution: a piece of *Tubularia* or *Corymorpha* stem so short that it gives rise only to a hydranth or the apical part of a hydranth under natural conditions may, under inhibiting conditions, develop a complete hydranth on a smaller scale and stem, or in *Corymorpha*, hydranth, stem, and basal holdfast region (pp. 344-49). A similar decrease in scale of organization results from inhibiting conditions in planarian reconstitution (pp. 349-54). The evidence indicates that in hydroids and planarians the decrease in scale results from depression and decrease in length of a quantitative physiological gradient, and at present convincing grounds for regarding the decrease in echinoderms as fundamentally different do not appear. The small, thin-walled "neck" connecting ectoderm and entoderm in many exogastrulae is evidently a reconstitution, and its possible significance is an interesting question.¹⁶ It is apparently ectodermal in origin; and after return to water the entoderm often separates from it, leaving it attached to the ectoderm. Usually, if not always, it is a secondary modification, not a direct effect of the primary inhibition. Since it develops from what would have been the anal region in an entogastrula, it might be regarded as a proctodeum, developing under the experimental conditions though absent or not developing appreciably in the entogastrula. On the other hand, if the attached end of the entodermized ectoderm is physiologically apical, it might perhaps be regarded as representing more nearly a stomodeum induced by the entoderm, particularly in the forms with radial ectoderm and basal ciliated band, in which the flattened basal ectoderm seems to be more or less like the normal ventral region in certain respects (Fig. 91, *B, C*). It shows the highest rate of dye reduction after return to water (Child, 1936*b*), and the presence of the ciliated band about it suggests that it approaches the ventral region in physiological condition.

¹⁶ Fig. 90, *B, C, D*; Fig. 91, *A, B, C, E, G*.

However, decision between proctodeum and stomodeum is neither possible nor necessary. The neck is apparently a secondary ectodermal modification resembling an everted proctodeum or stomodeum.

In partly entodermized forms there is also entodermal reconstitution; entodermization is itself a reconstitution. The original prospective entoderm may form only a small part of the total entoderm, much less than under natural conditions, and probably in some cases it is completely dissociated; but development, either of entodermized region together with original entoderm, or of the former alone, is definite and orderly and apparently entodermal in character. Three entodermal regions, separated by constrictions, often develop secondarily; or after greater inhibition, only two, sometimes with a very small third region at the tip; or with still more inhibited development there may be no regional differentiation. Supposedly, the three or two regions correspond to entodermal regions differentiating in normal development; but if this is so, how is the regional differentiation determined in an external entoderm consisting in part of original entoderm, in part of entodermized ectoderm, or entirely of the latter? From the data available, it appears that regional differentiation of entodermized ectoderm does not occur unless some ectoderm remains, and it may be absent or almost absent if the ectoderm is very small (Fig. 91, *H*); but whether absence of entodermal differentiation indicates absence of some determining or inducing action of ectoderm, or is merely the result of extreme inhibition, is at present not known. In any case, differentiation of entodermized ectoderm with or without original entoderm and without invagination suggests orderly and definite interrelations between parts, even in the differentially modified forms.

Entoderm which has lost its epithelial character during exposure to lithium or other agents may, if exposure is not too long, regain epithelial order to some extent during recovery in water. For example, as noted above, forms like *A* and *B* of Figure 94 may become exogastrulae like *E-H* of Figure 91. That there is recovery of epithelial character in these cases seems evident. Moreover, after extreme inhibition spherical entodermal vesicles often appear free in the blastocoel during recovery; examples are shown in Figure 96. Individuals in which the whole basal region has become merely a cell mass with more or less dissociation (Fig. 96, *A*) may, after return to water, attain the condition of Figure 96, *B*, with large external epithelial sac, many dissociated cells in the blastocoel, and one or more epithelial vesicles. Many individuals like Figure 96, *C*, do not recover, but 20-25 per cent may attain the condition of Figure 96,

D or *E*. In *D* there is apparently re-ectodermization of the apical region, but *E* appears to be completely anaxiate; and it may perhaps be questioned whether the external epithelium retains entodermal character or has become ectoderm; it has undergone the decrease in thickness characteristic of ectoderm.

When entoderms of individuals in the condition of Figure 96, *A* and *C*, come into contact, they tend to stick together;¹⁷ and large masses, consisting of many individuals, often result. In these the entodermal regions in contact gradually dissociate into the interior of the mass, and the parts directly exposed to water become a continuous epithelium with the

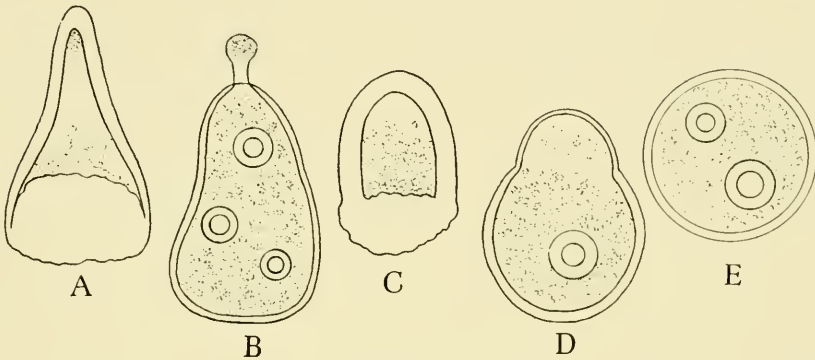


FIG. 96, A-E.—*Dendroaster*, recovery after extreme inhibition. *A*, *B*, LiCl m/20, 9 hr. from two-cell stage: *A*, 17 hr. water, *B*, 40 hr. water. *C*, LiCl m/20, 26 hr. from first cleavage. *D*, *E*, LiCl m/20, 26 hr., 24 hr. water.

apical regions at first protruding from the surface but gradually becoming incorporated into it, so that finally a large vesicle full of cells results, with complete loss of individuality of the component members. Within these masses, however, numerous epithelial vesicles may develop after return to water; they often persist free in the water after disintegration of individuals or multiple masses. The multiple forms are interesting cases of a sort of reconstitution with more or less complete obliteration of axiate pattern of the original individuals composing it and development of a new individuality in which there is no evidence of anything but surface-interior pattern.

Secondary reconstitucional modifications of skeletal development in

¹⁷ Less extremely inhibited exogastrulae often stick together by their entoderms if there is loss of epithelial character at the tip, but they do not lose their individuality so completely as the forms in which axiate pattern is almost obliterated.

echinoids present a wide range of variation, with aberrant localizations, deficiencies, excess of skeleton, and development of structures widely different from those characteristic of the species under natural conditions. Only a few of these are indicated in the figures.

The definite and orderly character of the reconstitucional changes in localization and differentiation of parts in exogastrulae represents the realization of potentialities in relation to an axiate pattern, modified primarily by differential inhibition and secondarily by differential tolerance, conditioning, or recovery. It is also evident that differential inhibition may completely obliterate axiate pattern, ventrodorsality being obliterated with less extreme inhibition than polarity. With increasing degree of obliteration there is progressively less evidence of localization and differentiation of particular parts, and with complete obliteration the individual remains completely anaxiate in development and without any regional differentiation. Particular features of axiate pattern are not fixedly associated with particular regions of egg or embryo but may be shifted in position with the experimental alterations of the pattern. At present it seems difficult to account for all the results of experiment otherwise than in terms of a primarily quantitative gradient or differential pattern involving metabolism, within which new gradients and specific differences gradually arise. When this pattern is altered differentially by external factors, the region of the embryo or larva in which a particular differentiation takes place may be shifted in one direction or another; or if the alteration is sufficient, the differentiation does not appear.

OTHER EXPERIMENTS AND INTERPRETATIONS

The early experiments of Herbst on the effects of artificial sea water, with certain salts or ions increased in amount, lacking, or replaced by others, were primarily attempts to determine what substances were necessary for sea-urchin development.¹⁸ They produced various modifications of development, including exogastrulation, but these were described as effects of particular experimental environments. Many of them are similar to the differential modifications discussed in preceding sections of this chapter, but the possibility that development might be altered differentially in similar ways by many environmental factors or that secondary modifications, opposite in direction to the direct effects of external agents, might occur after a primary inhibition, seems not to have been recognized. The significance of many of the modifications described and figured is,

¹⁸ Herbst, 1892, 1895, 1896*a*, 1897, 1901*a*, 1904.

therefore, uncertain; but it is evident that many of them are differential inhibitions. It is possible, however, that in some cases a modification resulting from absence of a particular constituent of sea water may be more or less specific in character, but to prove this requires extensive experiment—it must not be taken for granted. Herbst was concerned with other problems than those of developmental pattern, but many of his data point to the same conclusions as those of this chapter.

The attempts to interpret differential modifications of sea-urchin development, including exogastrulation, in terms of a single apicobasal gradient are, in the light of more recent work, applicable only to the earlier stages preceding gastrulation and to the ectoderm of later stages.¹⁹ The change in physiological condition of prospective entoderm and mesenchyme was not known at the time of these papers; and, though evidences of it were observed in some cases, their significance was not recognized, nor was dissociation of prospective entoderm actually observed. As regards the ectoderm, however, the interpretation suggested is essentially the same as that in this chapter.

Runnström's concept of two opposed and overlapping gradients in the apicobasal axis of the sea-urchin egg was originally stated in terms of concentration gradients of "animal" and "vegetal" substances, but more recently it has been maintained that specifically different animal and vegetal metabolisms characterize the axis. The hypothesis of two overlapping gradients was stated as if established fact in the introduction to the first of Runnström's experimental studies on sea-urchin development (1914) and has appeared in many papers since.²⁰ According to Runnström and Lindahl, lithium inhibits the kind of metabolism characterizing the animal gradient and stimulates or in some way favors that of the vegetal gradient. As already pointed out, differential susceptibility to gradually lethal agents, differential modification of development, and differential dye reduction give no information concerning presence or absence of such overlapping gradients. Moreover, it may be noted that two overlapping concentration gradients of different substances may be associated with a single gradient of metabolic rate; and metabolism, rather than concentrations of substances, is the effective factor in development of organismic pattern. Under natural conditions concentration gradients do not appear to be the primary factors determining metabolism; metabolism is unques-

¹⁹ Child, 1916*b*; MacArthur, 1924, accepting Child's views.

²⁰ Runnström, 1914, 1915, 1925*a*, 1928*a*, *b*, *c*, 1929*b*, 1931, 1933, 1935*a*, *b*, etc.; Hörstadius, 1928*b*, 1931, 1935, 1936*a*, *b*, 1937*a*, 1938; Lindahl, 1932*a*, *b*, 1933, 1935, 1936.

tionably an essential factor in establishing them. Differential modifications of development indicate a single primary activity gradient in the apicobasal axis and a secondary gradient arising later in the basal region and partly obliterating the primary gradient. Analysis of developmental stages of exogastrulae gives further evidence in support of this conclusion. Prospective entoderm is less susceptible than ectoderm in early stages, and more susceptible in later stages, to lithium and other agents. Lithium and various other agents entodermize prospective ectoderm in early stages, apparently by depressing or inhibiting it, but, after a certain developmental stage, inhibit entoderm more than ectoderm; that is, their action is inhibitory throughout. These experiments do not prove the absence of the gradients postulated by Runnström but indicate a different sort of gradient pattern as the primary effective factor in determining form and proportions, and alterations of form and proportion are often associated with changes in localization and differentiation of parts, indicating that these factors of development originate in the same pattern. Evidences of specific regional and local differences certainly become increasingly evident as development progresses, but it does not necessarily follow that they are the primary features of pattern.

Runnström has used potassium-free sea water, ZnSO_4 , lithium, CO, and other agents in modifying echinoid development; but determination of the effects of a wide range of concentrations of the chemical agents and of different exposure periods at different stages of development does not appear to have been undertaken to any great extent. Apparently, neither the possibility of change in susceptibility of prospective entoderm in the course of development nor that of secondary developmental modifications, opposite in character to the primary, in low concentrations of external agents or in recovery after return to water, has been recognized. Consequently, it is often difficult to determine whether forms described are direct effects of the agents used or secondary modifications. The experiments with potassium-free sea water suggest several possibilities: distribution of potassium may differ regionally in the egg or embryo; with lack of external potassium internal distribution may be altered; and the effect on development of absence of an essential ion, such as potassium, may be different from that of a chemical or physical agent acting in addition to the natural environment. Experiment with different amounts of potassium, both below and above the content of natural sea water, appear desirable as an adequate basis for analytic interpretation.

In several papers Lindahl and others have presented voluminous experi-

mental data on developmental modification and metabolism in the sea urchin. They have described effects of various agents and of absence of the sulphate ion and from these experiments have drawn certain conclusions concerning metabolism.²¹ As regards some of this work, it is difficult to resist the impression that the experimental data do not always provide an entirely adequate basis for the hypotheses advanced. Only a few points of these investigations can be touched on here. Accepting Runnström's hypothesis of two opposed, overlapping gradients, Lindahl and his co-workers present various lines of evidence which they regard as justifying the conclusion that the metabolisms of animal and vegetal regions differ specifically in character. Certain agents are believed to "animalize," that is, to increase and extend the animal kind of metabolism; certain others, to "vegetativize" (vegetalize). Here, also, the possibility of differential tolerance, conditioning, or recovery is apparently not recognized, although it is noted incidentally that further modification of form may occur after return to water.

Perhaps the most interesting of the modifications described are the animalized larvae obtained by exposure of eggs before fertilization to NaSCN in calcium-free sea water, also to NaI and sometimes by exposure to calcium-free sea water alone, with fertilization and development in natural sea water. The animalized larvae are without entoderm or mesenchyme, that is, entirely ectodermal; and in the more extreme types the long cilia normally appearing as a group or tuft about the apical pole in certain earlier stages develop over much or all of the surface of the blastula-like forms (Fig. 97). The cells bearing the long cilia also become different from the general ectodermal epithelium and similar to cells of the apical tuft. These forms do not develop beyond the stage of ciliated blastula-like larvae, though they lose their long cilia in later stages. This modification is prevented by exposure to cyanide or to lithium after fer-

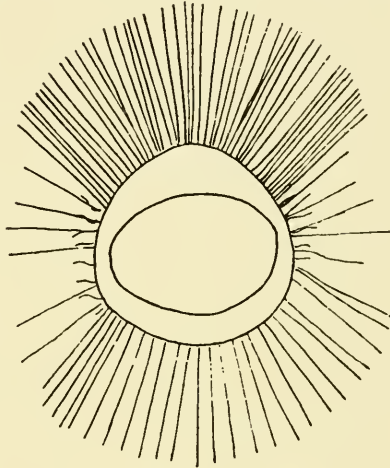


FIG. 97. *Paracentrotus lividus*.—In isotonic NaSCN (5 cc. NaSCN, 100 cc. sea water) for 18 hr. before fertilization; fertilization and 32 hr. development in sea water (from Lindahl, 1936).

²¹ Lindahl, 1933, 1935, 1936; Lindahl and Stordal, 1937; Lindahl and Öhman, 1938.

tilization; and with lithium, exogastrulae may develop from eggs treated with thiocyanate before fertilization. According to Lindahl, some of these exogastrulae are bipolar forms united by the distal ends of their external entoderms.²² The possibility that the animalized forms may not be direct effects of the thiocyanate or iodide but secondary modifications resulting from recovery after return to water, together with the activation associated with fertilization, is not considered by Lindahl. Some degree of ectodermization or animalization may apparently occur following exposure to lithium after fertilization (pp. 227, 235), and the possibility that Lindahl's animalized forms are cases of recovery cannot, at present, be excluded. The fact that cyanide and lithium after fertilization prevent the animalization of eggs treated with thiocyanate before fertilization supports, rather than conflicts with, the view that the animalization represents a recovery, not a direct effect of thiocyanate or iodide. Lindahl finds, also, that temporary exposure to thiocyanate or iodide after fertilization animalizes in earlier, vegetalizes in later, stages. This is what might be expected with differential recovery following inhibition.

Following Lindahl's procedure, Rulon (1938, 1940) has obtained animalized forms of *Dendraster*, though less extreme than some of those described by Lindahl for other species, and suggests that they represent recovery from a primary inhibition. According to this suggestion, thiocyanate inhibits differentially the slight apicobasal gradient present in the unfertilized egg, so that any dominance of higher gradient-levels or any definite relation between parts is almost or quite abolished; that is, in extreme cases axiate pattern is virtually obliterated. After return to water and fertilization, with the accompanying activation, the regions which would have been the lower gradient-levels and would, therefore, have developed as entoderm are more or less physiologically isolated, since they are in the same, or almost the same, physiological condition as more apical levels. Under these conditions they develop as higher gradient-levels. This may be regarded as essentially a reconstitution similar in principle to reconstitution of an apical region by a physically isolated basal half of a sea-urchin embryo and to reconstitution of apical regions by physically isolated pieces of hydroids and planarians.

²² In Lindahl's figures the supposedly bipolar exogastrulae are identical with cases of union of two exogastrulae by the tips of their entoderms. These appear in large numbers with the more extreme lithium inhibitions, and, as described above (p. 239), many individuals may stick together in this way with gradual obliteration of their individuality. At present it seems not impossible that the "bipolar" forms figured by Lindahl are not actually bipolar, in the sense that they have developed from a single egg, but are two exogastrulae united by the tips of their entoderms. In these unions the blastocoels become continuous by dissociation of the cells in contact, exactly as described by Lindahl.

Moreover, Rulon finds that, even with concentrations of thiocyanate and exposure periods preceding fertilization which produce the highest percentages (30-40) of ectodermized or animalized forms, the forms in a given lot range from these through normal individuals to differentially inhibited modifications. Also, with increase in concentration or exposure period preceding fertilization, frequency of animalized forms decreases and that of differential inhibitions increases. If animalization were a direct effect of thiocyanate, increase in frequency of animalized forms up to 100 per cent, or nearly, would be expected with increase in concentration and exposure, at least up to a certain limit. That is what happens as regards frequency of exogastrulation produced by lithium. But after thiocyanate, even in the most favorable cases, less than half the individuals exposed are animalized; and with increase in thiocyanate action beyond this point animalization is progressively decreased, and modification in the opposite direction, differential inhibition, increases. This is an effect exactly similar to the relations between differential recovery and differential inhibition with various other agents and stages after fertilization as concentration or exposure increases. Another point suggesting that the direct effect of thiocyanate is differential inhibition before fertilization as well as after is that in the more extreme animalizations ventrodorsality is apparently completely obliterated, though more or less polarity may still be present. Obliteration of ventrodorsality with polarity still an effective factor in development is a characteristic result of certain degrees of differential inhibition with various agents, as many of the forms in Figures 91-96 indicate. In the light of all the data available Lindahl's conclusion that animalization is a direct effect of thiocyanate treatment before fertilization seems not entirely satisfactory.

Experiments with sulphate-free sea water (Lindahl and Stordal, 1937), together with other earlier experiments, have led to the hypothesis that carbohydrate metabolism is characteristic of the animal region, protein metabolism of the vegetal. Only one line of evidence regarded as supporting this hypothesis is mentioned here. Development is found to be more modified in absence of sulphate than when it is present in the artificial sea water used; from this it is argued that in sulphate-free water the developing embryos give off more substance that inhibits development than when sulphate is present. Also, developing vegetal halves are injured by lack of sulphate, animal halves are not: on this basis the hypothesis is advanced that the vegetal halves produce poisons which are rendered non-toxic by sulphate. The possibility that absence of sulphate may itself inhibit development seems not to have been considered. Moreover, if the

vegetal region, that is, prospective entoderm and perhaps lower levels of ectoderm, undergo activation to a level above that of the apical or animal half at the time of gastrulation, as various lines of evidence considered in this and earlier chapters indicate, its greater injury by absence of sulphate may be due to increased susceptibility, as its dissociation by lithium apparently is; or after its activation it may produce certain metabolites in larger quantity than the animal half. The modifications figured and described in connection with these experiments apparently do not differ essentially from those produced by other agents. The hypotheses advanced may be entirely correct, but the evidence on which they are based does not appear adequate to exclude other possibilities.

Summing up, it appears that much of the evidence regarded as indicating presence of regional specificities in the sea-urchin egg and early embryo is open to other interpretations and that the data of differential susceptibility, both of differential death and differential developmental modification, and the data of differential dye reduction give no evidence of regional specificities. In fact, they indicate that such specificities are either absent in early stages or not sufficient to give rise to distinct, specifically different regional effects on development with different agents. Doubtless the metabolisms of ectoderm, entoderm, and mesenchyme do sooner or later become specifically different. Perhaps they begin to do so from the earliest stages of development or earlier; but, if so, the differences do not become sufficient to fix definitively their characteristics, that is, they do not become definitively "determined" until a later stage. Probably no one would maintain that all the local specific differences of later stages are present in the egg as actual, localized differences at the beginning of development: this, of course, would mean complete predetermination. But if new differences can originate and be localized during the course of development, the possibility cannot be excluded that specific differences of ectoderm, entoderm, and mesenchyme may not be present primarily or may be so slight at the beginning of development that they have little effect but increase gradually during development. In short, it appears possible, and much of the evidence supports the view, that specific regional differences arise secondarily from a primarily quantitative pattern. In early echinoid and asteroid development graded differences in rate of metabolism or of certain metabolic reactions appear to be much more important in determining the course of development and its modifications under experimental conditions than any regional specificities that may be present.

CHAPTER VII

DIFFERENTIAL MODIFICATION OF DEVELOPMENT: OTHER ANIMAL GROUPS

INVERTEBRATES

SLIGHT differential inhibition of larval development has been produced with a number of chemical agents in several species of polychete annelids (Child, 1917*d*). In these modifications apical and segment-forming regions are most inhibited, as might be expected from their greater susceptibility to lethal concentrations (pp. 120-29). However, the regions of the trochophore body are differentiated so early and the period from fertilization to swimming trochophore is so short in the species used that the alterations in form and proportions by external agents are not great, though they are clearly evident. Some modifications of later larval stages are apparently secondary, but they are so slight that their significance remains uncertain.

In reconstitution of pieces of oligochete annelids, particularly the microdrilous forms, physiological factors determining head frequency appear to be very similar to those in planarians. As Hyman (1916*a*) has shown, some microdrilous species regenerate a head at any body-level; in others head regeneration occurs only at levels near the anterior end of the body, irrespective of length of piece; and in still others, notably *Lumbriculus*, head frequency in pieces depends on level of body and length of piece, much as in *Dugesia* (= *Euplanaria*). According to Hyman, the physiological factor inhibiting head development in the shorter pieces of *Lumbriculus* results from the posterior section, as it does in *Dugesia*, and is a stimulation of the piece. Apparently, also, head development may be differentially inhibited in *Lumbriculus*. Hyman distinguishes microprostomial and aprostomial anterior ends and certain outgrowths apparently intermediate between head and tail or beginning development as one and undergoing partial transformation into the other. In pieces which give low head frequency in natural environment head frequency is increased by temporary exposure to an inhibiting agent (e.g., KCN), exactly as in *Dugesia*. This rather close parallelism in certain aspects of reconstitu-

tional development in planarians and annelids suggests existence of somewhat similar physiological patterns in the longitudinal axis. The problem of bipolar forms will be considered in a later chapter.

In several gasteropod species with free-swimming veliger larvae development of velum and shell gland have been inhibited by external chemical agents. In the inhibited forms the apical region remains smaller than in normal larvae, and the prototroch persists as a simple circular girdle of ciliated epithelium; even though the larvae remain alive for much longer periods than necessary for development of shell gland and spiral coiling

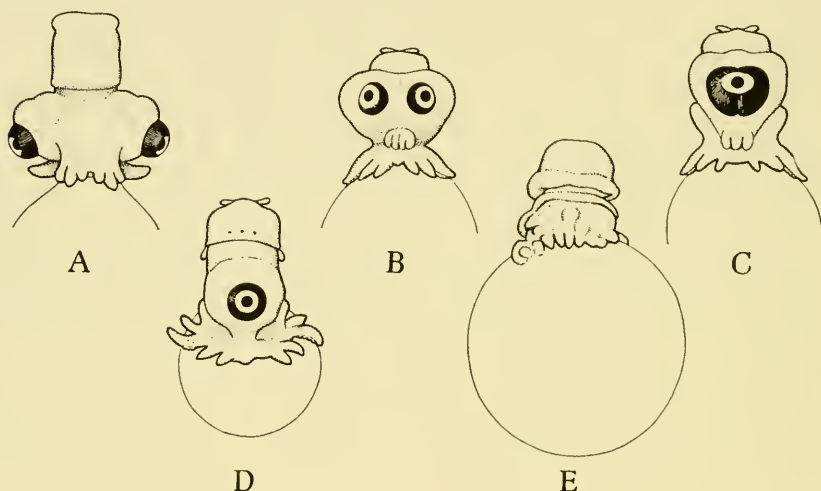


FIG. 98, A-E.—Differential inhibition in a cephalopod (after Ranzi, 1928, 1929a)

and velum, these may all be completely inhibited, the larva remaining in the primary trochophore stage (Child, unpublished).

Differential inhibition of development in the cephalopod *Loligo vulgaris* and other species has been described by Ranzi.¹ The modifications were produced chiefly by LiCl, although the author states that MgCl₂ and ultra-violet radiation produced similar effects. As with other forms, the individual modifications differ according to stage of development at which embryos are subjected to the agent, length of exposure period, concentration, and susceptibility of the individual. The most conspicuous feature of the inhibited forms is the differential inhibition of the head re-

¹ Ranzi, 1926; 1927; 1928; 1929a, b; 1931; 1932.

gion, resulting in all degrees of approximation of eyes to complete cyclopia, reduction in size of eyes, anophthalmia, and acephaly (Fig. 98, *A-E*). Evagination, reduction, or absence of the stomodeum also results. Other organs are also inhibited in many cases.

Ranzi interprets cyclopia in the squid as indicating a primary median position of the optic primordium, and the graded series of forms between cyclopia and normal position of eyes as different degrees of inhibition of its duplication and change of position, but there is no evidence in normal development of a primarily median and single optic primordium. It appears more probable that here, as in the planarian head, susceptibility decreases from the median region laterally, so that the median region is most inhibited; and with increasing degree of inhibition eyes develop nearer the median line or a single median eye forms. Moreover, the arms of the sea-urchin pluteus show a graded series of approximations to the median plane and a single median arm, and finally ventrodorsality is obliterated. It is certain that the arms do not develop from a primordium primarily median, but they show the same series of approximations to the median plane as the eyes of the squid and the planarian.

The hypothesis is advanced by Ranzi that differential susceptibility has nothing to do with quantitative physiological or metabolic gradients but that the most susceptible regions are those in which more complex embryogenic processes are going on, and that these regions are more susceptible only while these processes are occurring. What he means by "more and less complex processes" is not at all clear. If morphological complexity is meant, there is abundant evidence that his hypothesis is incorrect. The apical region of an alga axis is certainly, if anything, less complex morphologically than other parts, but it is more susceptible: the apical region of the sea-urchin embryo does not appear to be any more complex morphologically than other parts but is more susceptible. The regenerating planarian head is more susceptible than parts posterior to it and reduces dyes more rapidly in low oxygen from the beginning of its development on, as long as the animal is in good condition. If Ranzi²

² Ranzi, 1926, 1927, 1928, 1929*a*, *b*, 1931, 1932, 1938; Ranzi e Falkenheim, 1937, 1938. In several of these papers it is maintained that physiological gradient pattern either does not exist or is of no fundamental importance in development. Since the experimental evidence presented in these papers indicates presence of gradients, the views presented are personal opinions rather than conclusions from the data of experiment, and it need only be said that they are not in accord with a great volume of experimental evidence.

means physiological or chemical complexity, the hypothesis is merely an opinion without basis of evidence.

The only data on experimental differential modification of development in arthropods are apparently those obtained by Brauer (1938) on embryonic stages of a brucid beetle. In early stages (oviposition to $6\frac{1}{2}$ hours) axiate pattern can be completely obliterated by cyanide. In following stages ($6\frac{1}{2}$ –12 hours) susceptibility decreases anteriorly, posteriorly, and laterally from the presumptive prothoracic-maxillary region of the embryonic plate. In consequence of complete inhibition of the median region, more or less complete duplication may result by formation of new embryonic plates in the less susceptible and less inhibited lateral regions, which would give rise to lateral parts under natural conditions. Partial duplications of embryos show complete doubling in the prothoracic-maxillary region with incomplete duplication of heads and more posterior parts; complete duplications of all parts also occur (pp. 518–19).

ASCIDIANS

Very considerable differential modifications of development result from action of external inhibiting factors on early developmental stages of the ascidian *Corella willmeriana* (Child, 1927*d*). Developmental stages under natural conditions are outlined in Figure 99: the larva before hatching (*A*), the fully developed swimming larva (*B*), a stage of tail resorption (*C*), tail completely resorbed, with chorda cells aggregated in a mass (*D*). Development to the swimming larva takes place in the atrial cavity of the parent at a pH below that of normal sea water, and removal of eggs or early embryonic stages from the atrial chamber to sea water inhibits development differentially. Experiment indicates, however, that CO₂ content of water, rather than hydrogen ion concentration, is the important factor in the atrial chamber, the eggs apparently being conditioned to a higher CO₂ content than that of normal sea water. Sea water with alkalinity increased by NaOH is more strongly inhibiting than natural sea water.

The chief modifications thus far observed are indicated in Figures 99 (*E*–*G*), 100, and 101. In uninhibited larval development the tail always coils ventrally around the larval body (Fig. 99, *A*). With slight inhibition it may be bent in various directions (Fig. 99, *E*), but with greater inhibition it extends dorsally instead of ventrally and is shortened and folded or is represented by a rounded mass in which cells of the notochord are visible but without definite order (Fig. 99, *F*, *G*, and Fig. 100). The dorsal

region of the body is also decreased in size and flat instead of rounded; the tail appears to arise farther dorsally, the sensory vesicle and tail are closer together, and the papillae at the "anterior" end are more dorsal than in

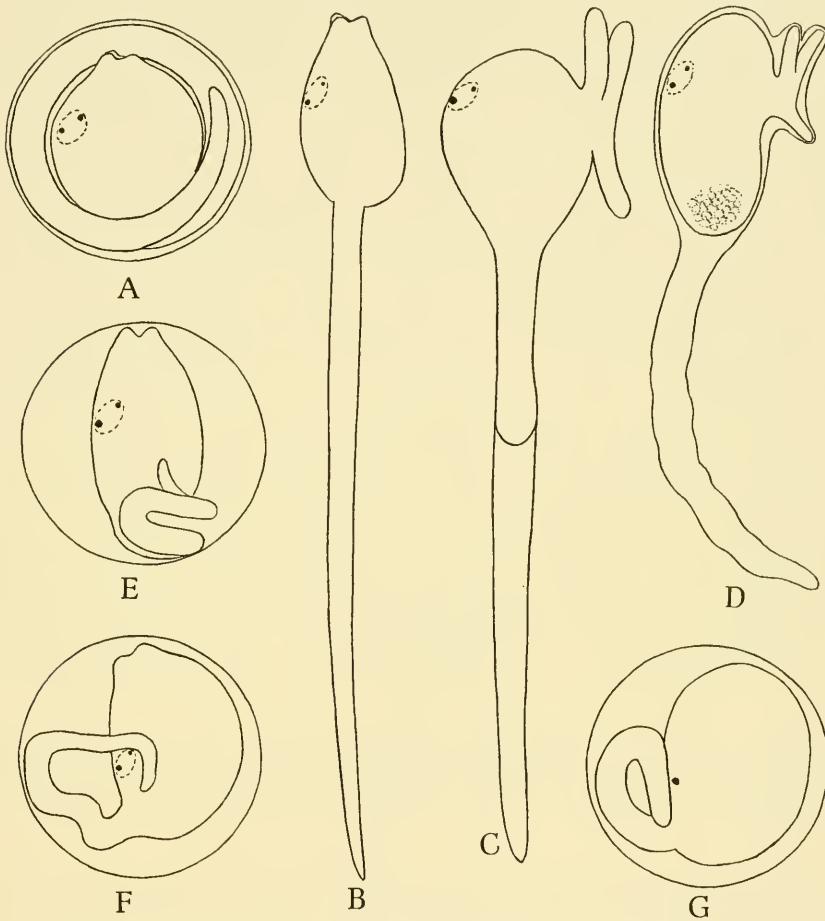


FIG. 99, A-G.—Uninhibited and differentially inhibited larval development of *Corella willmeriana*. A, uninhibited before hatching; B, fully developed larva; C, tail partially resorbed; D, tail completely resorbed; E, F, G, lesser degrees of differential inhibition (from Child, 1927*d*).

uninhibited individuals. Comparison of Figure 99, A and B, with Figure 99, E-G, less extremely inhibited forms, and with Figure 100, more extreme inhibitions, will show the differences. Differential reduction of KMnO_4 in developing larvae indicates the tail as the most intensely active

region, the dorsal region next; the developmental modifications show them to be the most susceptible regions.

In uninhibited individuals the sensory vesicle containing the two pigmented sense organs lies somewhat to the right of the median dorsal region. Among the more inhibited forms, individuals apparently completely bilateral, with pigment spots bilaterally localized at some distance

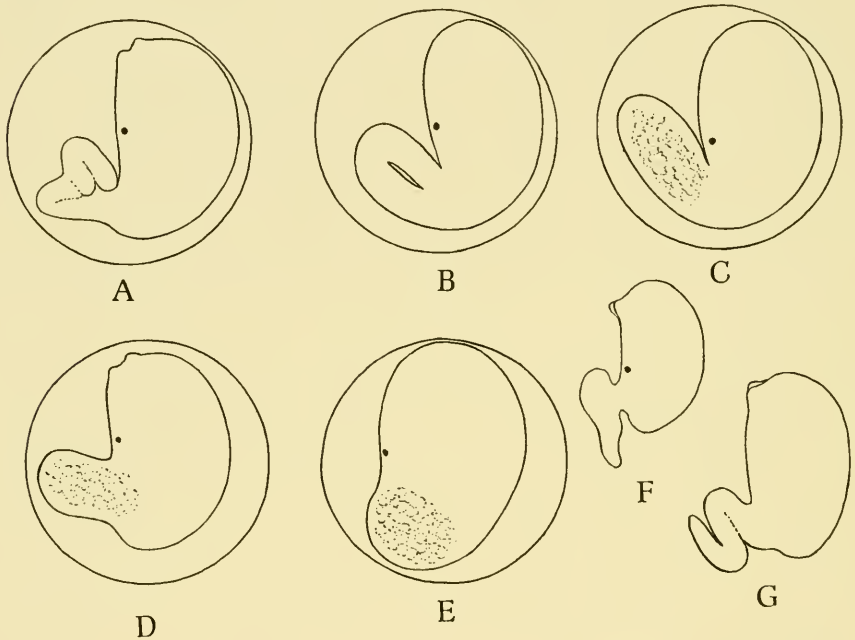


FIG. 100, A-G.—More extreme differential inhibitions of *Corella*; in all the most advanced stage of tail development is figured; all show dorsiventral differential inhibition (from Child, 1927 d).

from the median plane, appear rather frequently. The pigment spots are sometimes connected by a band of pigment like the eyespots of teratophthalmic planarian heads (Fig. 101). It would be of interest to know what sort of ascidian would develop from these apparently completely bilateral forms, but in all observed thus far there has been little or no development beyond the stages figured.

In uninhibited development the larval tail shows a very distinct gradient with high end at the tip. At or before the beginning of tail resorption this gradient disappears (pp. 145-47). With differential inhibition the caudal gradient is decreased or quite obliterated; consequently, the tail is smaller, shorter, or a mere cell mass containing chorda cells but without

definite pattern. The apparent association of orderly arrangement of chorda cells in a definite notochord with presence of a gradient in the

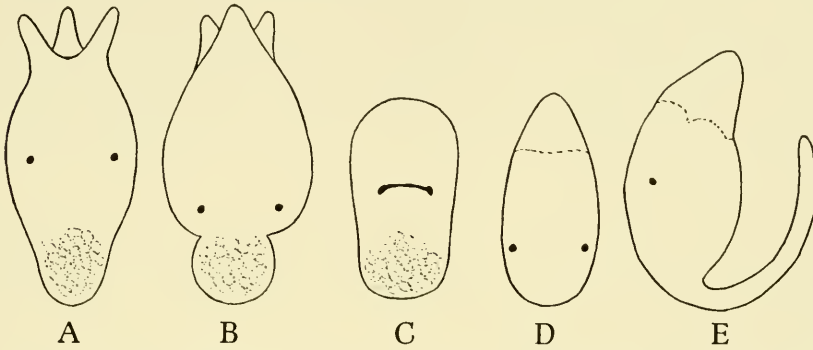


FIG. 101, A-E.—Bilaterally symmetrical forms of *Corella* resulting from differential inhibition (from Child, 1927d).

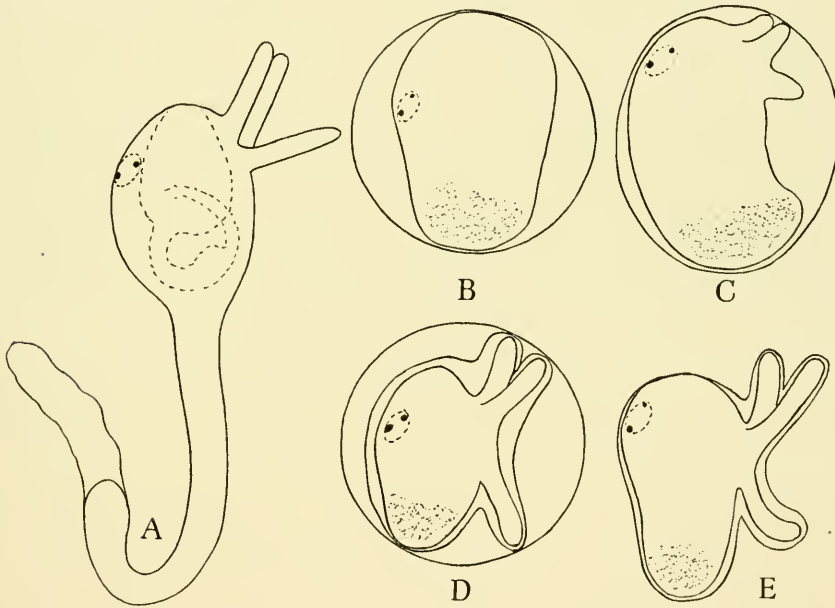


FIG. 102, A-E.—Stages of metamorphosis of *Corella*. A, uninhibited; B-D, differentially inhibited individuals metamorphosing without hatching; E, metamorphosis of somewhat inhibited individual after hatching but without development of tail at any stage (from Child, 1927d).

developing tail and loss of definite arrangement when the caudal gradient disappears is perhaps of interest.

The more inhibited individuals very commonly attain more or less advanced stages of metamorphosis within the egg membrane (Fig. 102) and,

if not too much inhibited, may become young ascidians before hatching. Developmental stages after resorption of the tail are much less susceptible than those of larval development; and differentially inhibited larvae in which a tail never develops may become apparently completely normal ascidians, though many of them fail to develop beyond early stages of metamorphosis. Much inhibited forms sometimes hatch but are incapable of movement, or the aborted tail may show only slight muscular tremors (Fig. 100, *F*, *G*).

VERTEBRATES

Extended consideration of the enormous literature of vertebrate teratogeny and teratology is quite beyond the present purpose. We are primarily concerned here with developmental modifications, apparently differential in character, that is, not specific for particular agents, and resulting from controlled exposure of the entire organism in early stages of development, or of egg or spermatozoon, to the action of physical and chemical agents or conditions outside the range of so-called "normal environment." These experimental modifications throw some light on the problem of origin of certain of the "accidental" teratological forms resulting from uncontrolled and unknown conditions. One of the most interesting results in this field of experimental vertebrate teratogeny is the high degree of similarity of teratological forms produced by different external factors. Although various authors have, from time to time, regarded terata produced by certain agents as specific for these agents, further experiment has shown that the supposed specificity did not exist. In the more advanced developmental stages after a considerable degree of differentiation particular agents may act more or less specifically on particular organs; but in the earlier stages less, or no, evidence of such localized specific action appears. If the yolk content differs greatly in different regions of the egg or embryo, as in amphibians, the yolk-laden parts may be more susceptible to certain agents and less susceptible to others than those with little yolk. This, of course, represents a specific difference resulting from regional differentiation already present in the egg. However, aside from such differences as these, the general similarity of the modifications produced by many different agents is evident, even in the work of the earlier investigators in this field,³ and some of them called attention to it. The evidence from later work supports the view that the modifications of early stages of vertebrate development by external agents depend

³ See, e.g., Dareste, 1891; O. Hertwig, 1892, 1895, 1898; Gurwitsch, 1895; Bataillon, 1901, 1904; Rabaud, 1901-2.

primarily on a differential susceptibility of different embryonic region, which is, to a high degree, similar for different agents though the differential in effect may be greater with some than with others. In many cases, however, analytic interpretation of the experimental data is difficult or impossible because of experimental procedure. The range of concentration or intensity used is often narrow; exposure to the agent is sometimes continuous, sometimes temporary, and, in certain experiments, repeated at intervals, and effects of exposures beginning at different developmental stages are not always clearly distinguished. The possibility of differential tolerance or conditioning to low concentrations or intensities and of differential recovery after temporary exposure has received no attention from most authors. In most of the experiments with earlier stages susceptibility decreases from the prospective anterior region posteriorly, but sooner or later a second region of high susceptibility appears at a more posterior level. In the fishes this occurs earlier in some species used in experiment, later in others (see Fig. 52, p. 149). In the amphibians the dorsal lip region has become highly susceptible at the beginning of gastrulation (Fig. 53, p. 152), and in the chick the region of the primitive streak from early stages to its disappearance is highly susceptible (Hyman, 1927a). Differential inhibition of the earlier stages always involves the head region to a greater or less degree; but modifications resulting from inhibition of the region of gastrulation may be absent, differ widely in degree, or appear at different body-levels, according to stage at which exposure begins, period of exposure, and rapidity and degree of action of agent, and possible individual differences in susceptibility. A variety of modifications may result from differential inhibition in a single experimental lot; and, if secondary modifications of conditioning or recovery are possible, the range of forms becomes still greater. Some have maintained that there is no discernible law and order in the teratogenic action of external agents (e.g., Kellicott, 1916; Cannon, 1923). As a matter of fact, however, law and order are becoming increasingly evident as experiment and analysis continue; but it is only by working with a considerable range of concentrations or intensities, exposure periods, and developmental stages that we can hope to attain definite results.

Numerous agents have been used in experimental vertebrate teratogeny: for example, magnesium salts, lithium salts, cyanides, inorganic and organic acids, bases, various other electrolytes, anesthetics, alkaloids, acetone, formaldehyde, low and high temperature, ultra-violet radiation, X-rays, and radium. In most experiments the agent used has been ap-

plied directly to developmental stages, but in some cases the effects on development of treatment of ova or spermatozoa preceding fertilization have been determined. In certain cases genetic factors have been found to be concerned in producing developmental modifications similar to those resulting from experimental conditions.

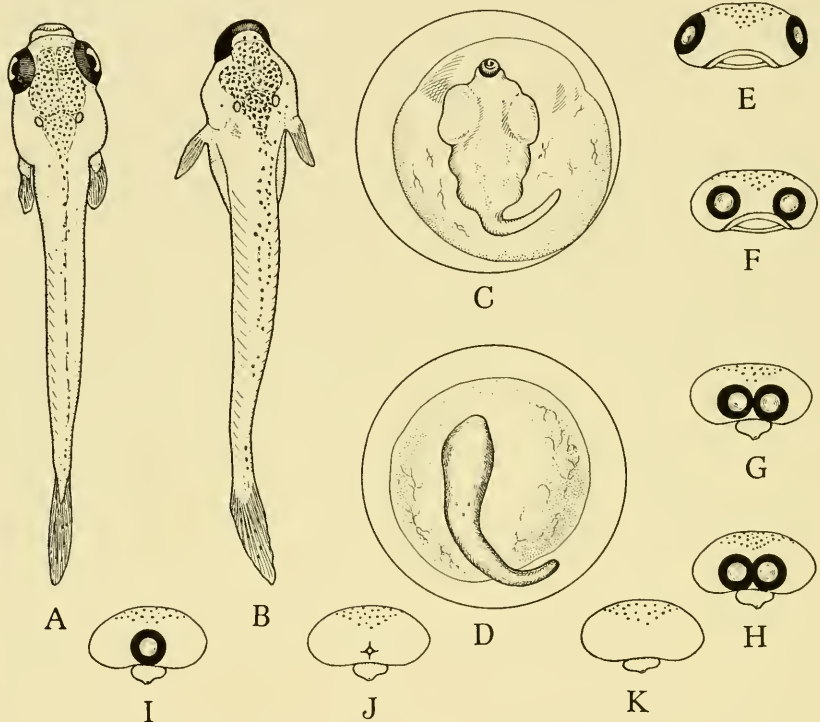


FIG. 103, A-K.—Differential inhibition of head in *Fundulus heteroclitus*. A, uninhibited; B, cyclopia; C, cyclopia with reduction in size of eye; D, anophthalmia; E-K, anterior views of uninhibited head and various degrees of differential inhibition (A, B, E-K, after Stockard, 1909; C, D, after Werber, 1916a).

EXPERIMENTS ON DEVELOPMENTAL STAGES OF FISHES

The most conspicuous and most discussed feature of differential inhibition in fishes is the mediolateral differential inhibition of the head.⁴ In the inhibited heads eyes develop in all degrees of approximation to the median line to complete cyclopia, reduction in size of the median eye, and anophthalmia (Fig. 103). In the more extreme cases there is usually reduction of the anterior brain region; but, according to Stockard, cyclopia

⁴ See, e.g., Stockard, 1907a, b, 1909, 1910a, 1921, and other papers; Lewis, 1909; McClen-don, 1912a, b; Werber, 1915, 1916a, b; Kellicott, 1916; Gianferrari, 1921; Hinrichs, 1925.

may occur without appreciable brain reduction. Stockard's interpretation of these modifications as indicating a median origin of the optic primordia and prevention of their later separation or of attainment of the usual position does not necessarily follow from the experimental data (see pp. 282-85).

In many of the modifications in fishes the head region is strongly inhibited; posterior regions, little or not at all, because exposure to the inhibiting agent was temporary and ended before the posterior region developed high susceptibility. In various other cases further experiment is necessary to determine whether differential conditioning or recovery may be concerned in the result. In the more extreme degrees of inhibition posterior regions are usually more or less inhibited or absent (Fig. 103, *C, D*). Development of the heart may also be differentially inhibited by external agents, so that it remains tubular; and a periodic reversal in direction of beat has been observed in certain cases (Gowanlock, 1923).

Asymmetric forms with a single eye on one side of the head have been described frequently. Probably many of these result from unequal exposure to the inhibiting agent or from unequal oxygen supply or unequal diffusion of CO_2 . If the developing embryos lie undisturbed on the bottom of a container or if floating eggs are in contact or aggregated in groups, such differences may arise. When exposure to the agent begins in early stages, difference in stage of the division cycle on the two sides of the blastoderm, with resulting difference in susceptibility at the time when the agent becomes effective, may determine later asymmetry. But whatever the factor concerned in a particular case, it seems to be incidental. Evidences of differential acceleration of development with production of megacephalic forms in *Macropodus* have been obtained by Gowanlock.⁵

EXPERIMENTAL MODIFICATIONS OF AMPHIBIAN DEVELOPMENT

In the many studies of modification of amphibian development different agents, concentrations, and intensities have been used, different developmental stages have been exposed and for different periods, and a great variety of forms has resulted. Most of these show the characteristics of differential inhibition and indicate the presence of a definite differential susceptibility pattern, that is, the modifications with different agents give little or no evidence of regional specificity.⁶

⁵ Unpublished. See Child, 1924b, p. 85, for figure.

⁶ The following references illustrate, to some extent, the development of investigation in this field: O. Hertwig, 1892, 1895, 1898; Gurwitsch, 1895; C. B. Wilson, 1897; Bataillon, 1901, 1904; Bohn, 1903; Morgan, 1903; Schaper, 1904; Jenkinson, 1906b, 1911a; Levy, 1906; Bardeen,

According to Bellamy (1919), who has given particular attention to the modifications of early stages, differential inhibition appears first in alteration of the "cleavage ratio," that is, a graded decrease in rate of cleavage,

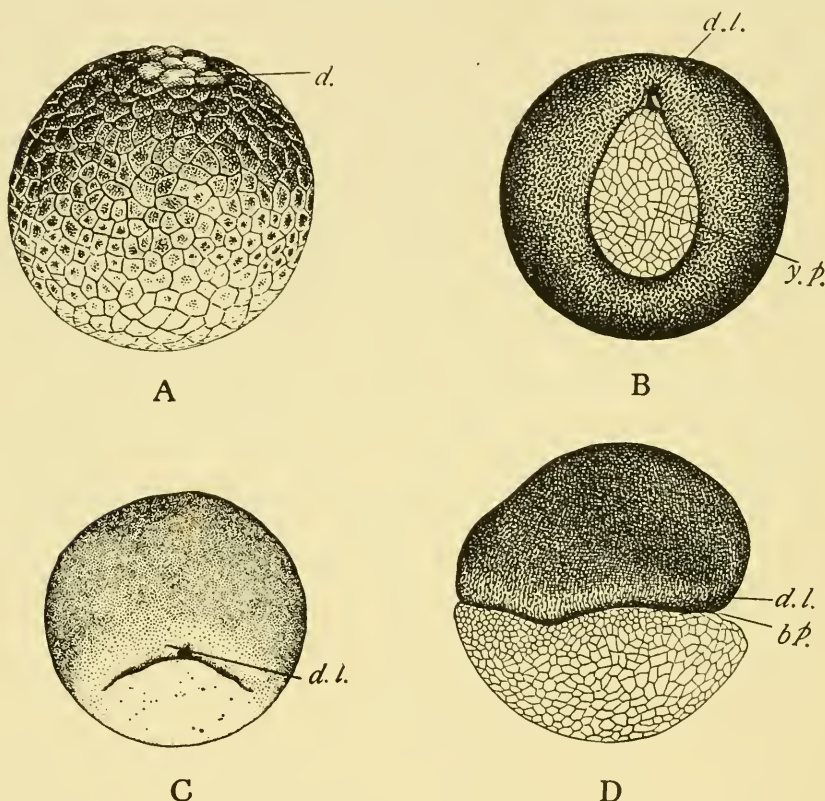


FIG. 104, A-D.—Differential inhibition in early development of frog (*Rana pipiens*). A, differential inhibition of cleavage, KCN m/1,000, 24 hr. from two-cell stage, then in KCN m/5,000, 24 hr.; B, differential inhibition of dorsal lip in gastrulation, median region most inhibited, LiCl m/10.62, 38 hr. from advanced cleavage; C, more extreme inhibition of gastrulation, median dorsal lip almost completely inhibited, lateral lips also retarded, LiCl m/10.62, 28½ hr. from one-cell stage; D, equatorial gastrulation resulting from differential inhibition, LiCl m/10.62, 76 hr. from one-cell stage (from Bellamy, 1919).

greatest apically and decreasing basipetally. With certain degrees of this inhibition blastomeres may be approximately the same size throughout (Fig. 104, A). Under inhibiting conditions so severe that development

1909; Doms, 1915; Baldwin, 1915, 1919; Bellamy, 1919, 1922; Leplat, 1920; Cotronei, 1921, 1922; Higgins and Sheard, 1926; Motomura, 1931; Holtfreter, 1934b; Lehmann, 1933, 1936a, b, 1937a, b, c, 1938.

stops in early cleavage, a few cleavage furrows may appear about the apical pole, evidently before the agent is fully effective; but their progress basipetally is soon stopped, and the basal region does not undergo cleavage at all. With certain fat-soluble agents, such as alcohol, this type of modification may perhaps result from a higher concentration in regions of higher yolk content or from a more or less specific action of the fat-soluble agent on this region.

Under inhibiting conditions permitting continuation of development to the blastula stage or later, the less susceptible yolk cells may proliferate into the blastocoel and in some cases completely obliterate it, the degree of such modification depending on concentration of agent and stage when exposure begins. It has been generally observed that inhibiting agents determine disturbances in gastrulation. According to Bellamy, the distance between apical pole and level of earliest stages of the blastopore decreases with inhibition. The median dorsal lip of the blastopore is most inhibited with the lesser degrees of inhibiting action; and, as gastrulation proceeds, the blastopore takes the form of an inverted U or V and later becomes ovoid in outline with its long axis in the median plane (Fig. 104, *B*). Under conditions severe enough to inhibit lateral, as well as dorsal, lips, the developing blastopore takes the form of a transversely flattened crescent (Fig. 104, *C*) and progresses subequatorially or even equatorially around the embryo (Fig. 104, *D*). In these individuals the blastopore makes its appearance near or at the equator, that is, much nearer the apical pole than normally; and the progress over the yolk of the blastopore lips may be completely inhibited, so that much or all of the basal hemisphere remains a permanent yolk plug. In some gastrulae of this type more or less elongation of the pigmented region in the apicobasal axis may take place in recovery, but dorsiventrality and bilaterality are apparently completely obliterated (Fig. 105, *A*). These equatorial gastrulae have not been observed to develop appreciably farther, even after return to water; probably their failure to do so is associated with the more or less complete obliteration through differential susceptibility of dorsiventral and mediolateral gradient differences. However, a secondary invagination often develops between the original blastopore and the apical pole and may extend partly or completely around the embryo. Its characteristics are similar to those of the primary invagination; it begins dorsally and progresses ventrally (Fig. 105, *B-D*). The possibility that the beginning of normal gastrulation involves some degree of physiological

isolation from apical dominance and that under inhibiting conditions a secondary isolation may occur nearer the apical pole than the primary and determine a secondary invagination is suggested by Bellamy. These modifications have been obtained with LiCl $m/10$, HgCl_2 $m/500,000$,

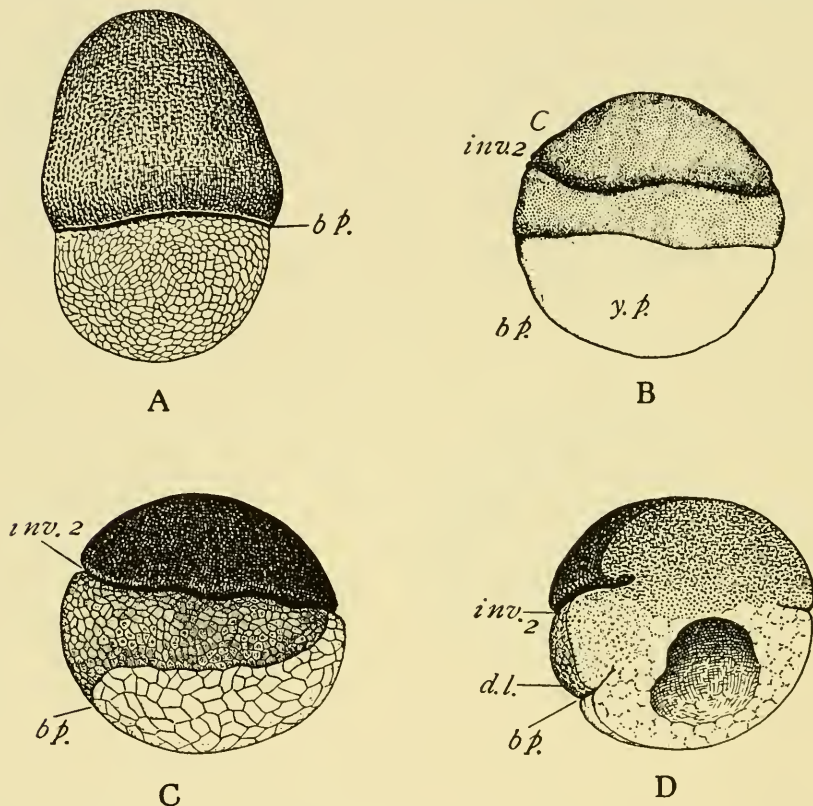


FIG. 105, A-D.—Differential inhibition in frog; gastrula stages. A, equatorial gastrulation and radially symmetrical embryo, LiCl $m/10.62$, 76 hr. from one-cell stage, water 20 hr.; B, equatorial gastrulation and secondary invagination nearer apical pole, HgCl_2 $m/500,000$, 24 hr. from one-cell stage, water 24 hr.; C, D, secondary invaginations, LiCl $m/10$, continuous from one-cell stage. *d.l.*, dorsal lip; *b.p.*, blastopore; *y.p.*, yolk plug; *inv. 2*, secondary invagination (from Bellamy, 1919).

0.001 per cent formaldehyde, and KCN $m/1,000$ with successive decreases in concentration after 24 hours.

Under experimental conditions permitting further progress of development not only do the resulting forms represent various degrees of differential inhibitions but with low concentrations or intensities unquestionable secondary modifications appear in later developmental stages.

Neurula stages with relatively slight inhibition show reduction of the anterior part of the neural plate and often a permanently open blastopore

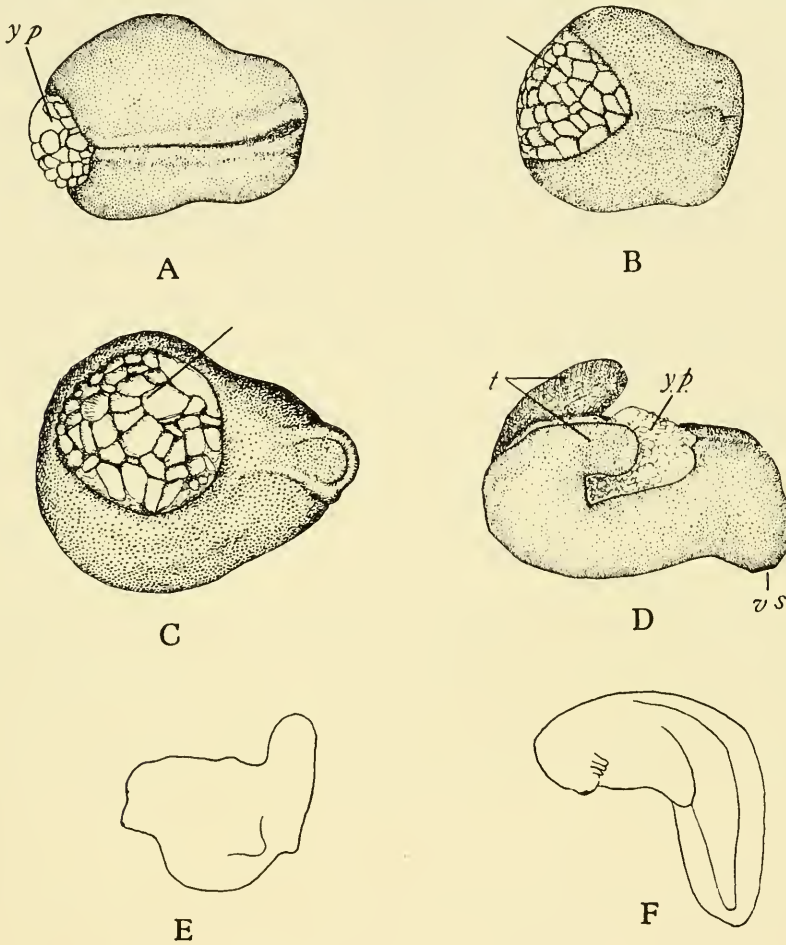


FIG. 106, A-F.—More advanced stages of differential modifications of frog development. A-E, differential inhibition. A, KCN, 4 days from two-cell stage, water 2 days; B, formaldehyde 0.0075 per cent, 2 days from four- to eight-cell stage; C, microcephalic with shallow neural groove and persistent yolk plug, KCN m/5,000, 24 hr., water 5 days; D, KCN m/10,000, 4 days, water 8 days; forms like A-D also obtained with LiCl; E, fused suckers and nasal pits, tail dorsiventrally differentially inhibited, LiCl m/5, 3 hr. at late cleavage, water 4 days; F, differential recovery, LiCl m/10, 2 days, water 5 days. *t*, tail; *v s*, ventral sucker; *y.p.*, yolk plug (A-D from Bellamy, 1922; E, F, from Bellamy, 1919).

varying in size. With increasing inhibition these characteristics become increasingly conspicuous (Fig. 106, A-C). With further development neural folds may fail to close posteriorly and be separated by the open

blastopore, giving various degrees of spina bifida, usually with microcephaly, sometimes almost complete acephaly. In cases of spina bifida two tails often develop, usually more or less bent dorsally, indicating greater inhibition on the more susceptible dorsal side (Fig. 106, *D*). This dorsal curvature of the tail or tails is highly characteristic of differential inhibition, even in absence of spina bifida (Fig. 106, *E*). All degrees of medio-lateral differential inhibition appear in the head region. Ventral suckers, olfactory pits, and eyes develop with increasing inhibition progressively nearer the median plane, may become single median organs, often with decrease in size, or may be entirely absent; and inhibited forms almost or quite acephalic occur. Since different regions of the embryo attain high susceptibility at different developmental stages, the forms resulting from differential inhibition differ according to stage and period of exposure to the agent. For example, with temporary exposure in earlier stages the head region may be greatly inhibited, the tail almost or quite normal, because the tail region was not highly susceptible at the stage of exposure. With temporary exposure at certain later stages the head may be little, the tail greatly, inhibited; but these differences apparently do not represent specific differentiations—at least they are not specific for particular agents.

The most conspicuous secondary modification with the less extreme degrees of inhibition in early stages is development of a relatively large tail bud with ventral curvature of the developing tail (Fig. 106, *F*), that is, greater elongation of the dorsal side, a modification opposite in character to that of direct differential inhibition (Fig. 106, *D*, *E*). These forms are frequent with return to water after temporary exposure and appear, beyond question, to be cases of dorsiventral differential recovery. They are less frequent with continuous exposure, but sometimes appear apparently with differential conditioning, though less extreme than after return to water. Animals inhibited to the spina bifida condition (Fig. 106, *A-D*) usually show little or no secondary modification; they may undergo some further development after return to water, but differential inhibition remains predominant.

With low concentrations of NaOH and of alkaline KCN and by addition of HCl to a well water with high carbonate content Bellamy (1922) obtained acceleration of development with indications of differential acceleration in precocious head development and megacephalic tadpoles (also Child, unpublished).

Forms described by Higgins and Sheard (1926), resulting from daily

1-minute exposures to ultra-violet radiation of a certain wave length range, show strong ventral curvature and are apparently cases of differential tolerance or conditioning, while those resulting from daily 2-minute exposures are, at least in part, differentially inhibited forms with persistent open blastopore.

Bellamy's experiments show that the dorsal lip of the blastopore at the beginning of gastrulation is highly susceptible to many agents and that its median region is more susceptible than lateral regions, but they do not indicate specific susceptibility to any of the agents used. Moreover, they do not show whether, or to what extent, ectodermal differential inhibitions of the head and dorsal region are direct effects of the agent on the ectoderm or results of differential inhibition of the chorda-mesoderm, the dorsal inductor (see chap. xii). The high susceptibility of the dorsal lip, decreasing anteriorly and laterally, suggests that differential inhibition of this region may be of primary importance in various modifications of the ectoderm, but doubtless direct differential inhibition of the ectoderm after induction has taken place plays a part in the modifications—for example, in those of the head.

By removal of membranes of axolotl and anuran blastulae and culture in a modified Ringer solution partial or complete exogastrulation has been produced.⁷ In total exogastrulation no neural plate develops in the ectoderm, apparently because of absence beneath it of the entomesodermal inductor, but the evaginated entomesoderm differentiates without evidence of retardation or differential inhibition (see pp. 463–65). Exogastrulation also occurs in some cases in water after removal of membranes, apparently in consequence of collapse of the blastocoel; but in salt solution all axolotl embryos become exogastrulae. From the data it appears probable that a differential susceptibility is not primarily concerned in this type of exogastrulation. The collapse of the blastocoel in the soft, highly fluid axolotl embryo without membranes is probably the chief factor.

The view that lithium has specific regional effects, differing at different developmental stages and dependent on critical stages or phases in development of the dorsal inductor region, and that action of external agents in general on embryonic development shows many evidences of specific regional effects differing at different stages has been advanced recently in a number of papers by Lehmann.⁸ The evidence presented in these papers, however, does not at present seem to provide adequate basis

⁷ Holtfreter, 1931a, 1933d, e; see also Goerttler, 1926.

⁸ F. E. Lehmann, 1933; 1934; 1936a, b; 1937a, b, c; 1938.

for the conclusion that the local differences in susceptibility to lithium or other agents constitute evidence for the presence of local specific differences in the embryo. Considering one case, for example, Lehmann finds that treatment with lithium at the beginning of gastrulation produces otocephaly with anterior head region normal, while lithium treatment at the midgastrula stage produces cyclopia, with otic region normal. The possibility that in the first case early invagination of the part of the dorsal inductor which attains later the most anterior position may protect it to some extent from lithium action does not seem to be considered. If invagination does protect in this way, Lehmann's results may be a matter of differential exposure to lithium rather than of specific regional difference. The possibility that different degrees of recovery may occur in different regions is also not considered. In the second case, lithium treatment at midgastrula stage, anterior head region, and otic region may be more or less equally exposed, and the more susceptible anterior region more inhibited. However, cyclopia may also result from exposure to inhibiting agents beginning in early cleavage stages.

Formation of notochord may also be inhibited by lithium treatment, according to Lehmann, the prospective notochord being "mesodermized" and incorporated into the somites, and these become continuous across the median plane. Continuity of somites across the median plane has been obtained by lithium treatment of late blastulae or early gastrulae of *Amblystoma* with retarded development, not absence, of notochord (Cohen, 1938a). In these individuals the notochord has evidently not been mesodermized, and transverse continuity of somites dorsal to it is apparently a matter of physical conditions resulting from retardation of its development. It is suggested by Cohen that in Lehmann's material the notochord is not mesodermized but is never segregated from the archenteric roof, that is, under the inhibiting conditions it remains entoderm. The modifications of anuran development, evidently differential inhibitions, obtained by Hoadley (1938) with high temperature, show similar inhibitions of notochord and median continuity of somites. Evidently this modification is not specific for lithium, and the fact that it is more conspicuous anteriorly suggests differential susceptibility in relation to an anteroposterior gradient in the invaginated chordamesoderm; the presence of such a gradient is shown by differential lethal susceptibility and by differential dye reduction and is also suggested by the different degrees of inductor action by different portions of the dorsal inductor region when implanted. Once more it may be emphasized that what may

appear, with a few experiments, to be regionally specific effects, may prove, on more extensive investigation, to be nothing of the sort. Lehmann's conclusions may be entirely correct, but the experimental evidence does not, at present, exclude other possibilities, and the developmental modifications concerned do not appear to be essentially different from others, apparently not specific. Further experiment with different developmental stages, different agents, and a wide range of concentrations and exposure periods is still highly desirable.

HIGHER VERTEBRATES

Experimental modification of development in the chick by subsection of the embryo to particular external agents and altered environmental conditions presents certain difficulties. When a chemical agent is applied inside the shell, there is no certainty that all embryonic regions are subjected to the same concentration at the same time; and when it is allowed to diffuse through the shell, the possibility of control of distribution is even less. Uniform decrease of oxygen supply to different parts of the embryo cannot be certainly attained by decreasing the surface through which oxygen can enter or by decreasing oxygen in the surrounding atmosphere, because differences in oxygen consumption of different embryonic parts may decrease the supply at very different rates about different embryonic regions. The only external factors which meet the requirements seem to be change of temperature and perhaps X-rays and radium. In view of this situation and of the possibility of differential tolerance, conditioning, and recovery it is often impossible to determine the significance of a particular teratological embryo. Nevertheless, the general similarity of many modifications of chick development to those of fishes and amphibia, which apparently depend on differential susceptibility, leaves little doubt that essentially similar physiological factors are involved in all three groups. More than forty years ago Dareste (1891) concluded that similar modifications are produced by different external conditions, that the same external factors do not always produce the same modifications, and that the teratological forms depend on time, intensity, and duration of action rather than on the nature of the external agent. Later work, in large part, confirms these conclusions. Hyman (1927*a, b*), discussing differential susceptibility in the chick, points out that the regions most modified are those most susceptible to lethal agents.

Inhibition of head development, approximation of eyes and other lateral cephalic organs to the median plane, their development as single median

organs, their absence, anencephaly, and acephaly all occur in the chick. The developing heart is, in its earlier stages, a region of high susceptibility and frequent, apparently differential inhibition. Decreased elongation, spina bifida of various degrees at various levels, and inhibition of posterior regions are, as Hyman points out, associated with the high susceptibility of the region of posterior elongation which becomes separated from the highly susceptible head regions with the progress posteriorly of the node and primitive streak. With sufficient inhibition the whole embryo may be absent.⁹

The early developmental stages of mammals are, as yet, scarcely accessible to long-continued experiment with altered environment. The effects on the nervous system of irradiation with X-rays and radium (Bagg, 1922) and of administration of alcohol to parents suggest embryonic differential susceptibility. In some of these experiments, however, the direct effect of the experimental conditions is on the germ cell rather than on the embryo; in all cases further experiment is necessary with data concerning early stages.

OTHER FACTORS IN MODIFICATION OF VERTEBRATE DEVELOPMENT

In his experiments on development of ligatured *Triton* eggs Spemann (1904) found that, when the ligature was slightly oblique to the median plane, more or less complete duplication of the anterior end resulted, as with median ligatures, but that the head developing on the side with less of the median anterior region may show all gradations of inhibition to complete cyclopia, the modifications being the same as the differential inhibitions produced by chemical and physical agents. In the light of more recent work of Spemann and his school it seems evident that the inhibition of head development is associated with inadequacy of induction, perhaps also with absence of the apical ectoderm.

Inhibition of development, apparently differential in character, following injury to the spermatozoon, is of particular interest because the injury, doubtless chiefly of the sperm nucleus, appears in development as inhibition with regional differential along one or more axes, as in cases of direct exposure of the ovum or developing embryo to an inhibiting agent. Eggs fertilized by spermatozoa irradiated by X-rays or radium, or ir-

⁹ For references to, and discussion of, the work of earlier experimenters—Feré, Windle, Kaestner, Mitrophanow, and others—see Hyman, 1926*a*. See also the following: Rabaud, 1901-2; Gilman and Baetjer, 1904; Tur, 1904; Reese, 1912; Stockard, 1914; Alsop, 1919; Riddle, 1923; Byerly, 1926; Buchanan, 1926*c*; Hinrichs, 1927; Gradzinski, 1933, 1934; also citations by these authors.

radiated ova fertilized by normal spermatozoa, have been found to give developmental modifications essentially similar to those resulting from direct action of inhibiting agents on developmental stages; treatment of ova or spermatozoa with chemical agents has given similar results.¹⁰ In the case of injured spermatozoa the effect of the injury on development is, in general, greatest in the regions most susceptible to inhibition by direct action of external agents, the regions of greatest developmental activity; that is, the cells of these regions with injured nuclei do not attain the physiological levels essential to full development, but development at lower gradient levels is less, or not at all, affected.

It has been shown that certain heterogenic hybrids, notably among the fishes, often show inhibitions of development similar in character to the differential inhibitions by external agents. In a series of papers concerned with fish hybrids particular attention has been called to this point by Newman.¹¹ He has noted in some detail the similarities of the hybrid terata to those determined by direct action of chemical and physical agents. In these hybrids development is retarded, and the monsters usually show what appear to be various degrees of differential inhibition, the heads being small, eyes approximated or cyclopic, the heart, and in some the posterior part of the body, inhibited as with external agents. Moreover, forms similar to cases of differential tolerance, conditioning, and recovery also appear. The similarity of hybrid monsters to modifications produced by external inhibiting agents was also noted by Loeb (1912, 1915). More recently similar terata have been reported by Montalenti (1933) as characteristic of the amphibian cross *Bufo viridis* ♀ × *B. vulgaris* ♂ but infrequent in the reciprocal cross.

Various suggestions have been advanced in attempting to account for the factors concerned in these modifications of hybrid development: toxic action of the sperm, in general more severe the less closely related the species crossed; nuclear "incompatibility" resulting in aberrant distribution of chromosomes or perhaps in inactivation or loss of chromosomes of parts of chromosomes; alteration of the nucleoplasmic ratio; asynchrony of developmental rate, particularly in the case of sperm from a species with slower development than the maternal species.

¹⁰ See, e.g., Opperman, 1913, fish spermatozoa, radium; Gee, 1916, fish spermatozoa and ova, alcohol, NaOH; Bardeen, 1907, amphibian spermatozoa, X-rays; O. Hertwig, 1911, 1913, amphibian spermatozoa, radium; G. Hertwig, 1911, amphibian ova, radium; G. and P. Hertwig, 1913, amphibian spermatozoa, nicotine, strychnine, chloral hydrate, various dyes; Stockard, 1912, 1913, guinea-pig spermatozoa and ova, alcohol.

¹¹ Newman, 1908, 1914, 1915a, 1917b, 1918.

Whatever the particular mechanism involved, it appears that in the hybrid monsters, as in those resulting from injury to the germ cells, the regions more susceptible to external inhibiting factors are, in general, more inhibited by these physiological factors which interfere in some unknown manner with the activities of life. The effect of the interference is primarily greatest in the regions most intensely active, but in some cases these regions apparently become more or less completely adjusted to the physiological interference or "outgrow" it. One is tempted to suggest that development of parts originating at high gradient-levels is more "difficult" than that of other parts; that is, for such development the mechanism must be in the best working order, and relatively slight interferences decrease its effectiveness more than that at lower levels, but the "best working order" also involves, within limits, the possibility of more rapid or more complete equilibration or adjustment to, or recovery from, the lesser interferences.

The enormous literature of descriptive teratology records numerous monsters, chiefly in man and other mammals, apparently representing all possible degrees of differential inhibition and perhaps in some cases secondary modifications. These have been variously classified and interpreted.¹² Little or nothing is known concerning the conditions determining these terata, but in the light of experimental teratogeny it is highly probable that many physiological or pathological factors may give rise to essentially similar modifications.

A large and extremely interesting series of monsters occurring in certain branches of an inbred strain of guinea pigs with relatively high frequency has been described and its genetic basis discussed by Wright and Wagner (1934; Wright, 1934). In this series a wide range of inhibited head forms appears with resemblances to experimental differential inhibitions, but most of them fall into two, rather than into a single, series. The chief forms are designated and defined by Wright and Wagner as follows:

Practically it turns out that a two-dimensional scheme includes nearly all of the combinations described. The mandible is regularly more susceptible than the maxillary process. This gives a basis for one series of grades.

Brachygnathus: Mandible short but of normal width.

Micrognathus: Mandible diminutive. Ventral approach of ears.

I'ypognathus: Mandible absent. Ear ossicles united.

Synzygo-agnathus: As above with more or less fusion of zygomatic arches.

¹² See, e.g., St. Hilaire, 1832-37; Ahlfeld, 1882; Ballantyne, 1904; Schwalbe, 1906-13, particularly Teil III, Abt. 1, Kap. 5 und 6, "Cyclopie, Otocephalie, Triocephalie, etc."; Abt. 2, Kap. 1, "Die Missbildungen des Auges," Kap. 2, "Missbildungen des Nervensystems," Kap. 5, "Missbildungen des Gebisses," Kap. 6, "Die Missbildungen des Ohres."

Azygo-agnathus: Zygomatic arch absent or vestigial. No mandible.

Premaxillary defect has no regular relation to this series, and the same is true of defects of the nose, brain and eyes but premaxillary defects always seem to occur with the latter group and these usually occur in a regular sequence of increasing susceptibility from anterior to posterior. This gives the basis for another series of grades. Most of the terms are familiar in the literature of the subject.

Brachyrhynchus: Premaxillary reduced or absent. Brain normal.

Arrhinencephalus: As above except defect of olfactory lobes and more or less union of cerebral hemispheres. Usually one nostril (monorhinus).

Rhinocyclops: Forebrain vesicular. Eyes more or less united. Proboscis above the single orbit.

Cyclops arhinus: As above except for absence of proboscis.

Anops: As above except for absence of eye.

Aprosopus: Cerebellum and anterior parts of brain absent.

Monoto-aprosopus: As above, ear vesicles united.

According to the interpretation offered by Wright and Wagner, different regions of the developing head attain their maximum susceptibility at different stages, and the character of the individual modification depends on the stage at which the inhibiting or depressing factor becomes effective. That genetic factors are concerned in these modifications has been demonstrated by Wright (1934). He suggests that they act by bringing about, directly or indirectly, a general depression of vital activity at a particular critical moment with permanent effects on the development at the anterior end of the polar embryonic axis as the most active, and hence the most susceptible, region at the time when the action takes place. The depressing action at an earlier stage produces different modifications from action at a later stage. The same is true for action of external inhibiting agents. But chance conditions also play a part in determining the individual forms; chance differences in implantation or in blood supply or chance mutations are suggested by Wright and Wagner as possible factors. "The randomness of occurrence within litters of each size indicates that each monster is due to a highly localized chance event, supplementing a genetic tendency common to all members of the group" (Wright, 1934, p. 502).

In an inbred strain of mice Little and Bagg (1924) obtained forms with reduced lower jaw and frequent microphthalmia, apparently representing somewhat less extreme degrees of differential inhibition than the guinea pigs. Occurrence occasionally in mammals of a single individual or a single litter showing characteristics of differential inhibition has been attributed to various factors—for example, to maternal toxemia associated with disease or other conditions—but practically nothing is known concerning the particular factors involved in many cases. Results of experi-

mental teratogeny lead us to believe that the determining factors may differ widely even though the modifications are similar.

Apparently inhibition in earlier stages may produce cyclopia in vertebrates; in later stages after normal localization of optic primordia, only microphthalmia. The occurrence of cyclopia has suggested that the optic primordium may be primarily median and become bilateral later and that the inhibiting conditions merely prevent the secondary change in localization. This question is discussed later in connection with certain transplantation experiments which bear upon it (pp. 282-85).

CONCLUSION

Differential modifications of development indicate presence of physiological factors of developmental pattern which are very similar as regards regional differences and relations in various animal groups, from coelenterates and flatworms to the higher vertebrates. The fact that a large number of external agents, both chemical and physical, within certain ranges of concentration or intensity, produce similar modifications of early development with axial gradations in degree of modification indicates that the physiological factors on which the modifications depend constitute a quantitative gradient pattern, rather than a regionally specific pattern of different substances and metabolisms. The possibility that a regionally specific pattern may also be present is, of course, not excluded by the characteristics of the modifications; but if it is present, it is evidently not the chief factor in determining the similar graded characteristics of the differential modifications. Moreover, the alterations in localization of particular differentiations and the complete obliteration of axiate pattern and all localized differentiation by differential inhibition and obliteration of the gradient pattern suggests that differentiations are dependent on, and results of, this pattern.

The various lines of evidence considered in this and preceding chapters indicate that axiate pattern is primarily a quantitative gradient pattern, involving the essential activities of living. Within this general pattern specific differences of substance and reaction arise and become the basis of differentiations. With the changes in activity in the course of development new centers of activity and new gradient systems may arise, some of them with metabolisms different in character from that of the primary pattern. As these differences increase, they affect activities in other parts, and physiological interrelations and integrating factors become increasingly effective and varied in character. Apparently, however, quantitative gradient factors may persist and be physiologically ef-

fective from the beginning of development to maturity in many of the simpler organisms and in some of the organ systems, even of vertebrates and man. Such factors become evident, for example, in the reconstitution of isolated pieces of hydroids, planarians, and many other forms; the localization of the reconstituting hydranth or head evidently depends on these gradient factors rather than on the specifically differentiated organs in the piece. Gradient factors are also evident in the functional gradients of many axiate organs—for example, the mammalian intestine (see p. 164).

In many animal eggs specific regional differentiations and metabolisms are apparently already present in the cytoplasm at the beginning of embryonic development, but quantitative gradient factors may also be present and effective. All that we know concerning developmental physiology indicates progressive increase of specificity of parts during the earlier stages: the progress of so-called “determination of parts” and of visible differentiation certainly constitute abundant evidence of this increase. By a sort of physiological extrapolation to successively earlier stages of development, to the ovarian development of the oöcyte, to the beginnings of reconstitution in the isolated piece, to the bud in its initial stages, and to the aggregate of dissociated cells we seem to find it necessary to postulate a quantitative gradient pattern as the primary axiate pattern, and many lines of experiment show the presence of such pattern. It is quite unnecessary, however, to assume that every individual organism begins its development with nothing but this primary gradient pattern. Obviously, this is not the case. The reconstituting planarian piece may contain various organs; its polarity usually determines on which end the head shall develop, and the ventrodorsality of the regenerated head is evidently derived from that of the piece, but the new gradient pattern resulting from activation and formation of new growing tissue at the anterior end makes over—reorganizes—the piece. All that we can learn concerning the organization of eggs at the beginning of embryonic development and the changes during the course of development is, of course, of value in the analytic investigation of that kind of development; but it should not be forgotten that this organization may be far from the primary developmental pattern and significant rather as a feature of a particular kind and stage of development than as representing fundamental factors of developmental pattern. We have already learned that various features of egg organization are results—effects—rather than essential factors of the real pattern.

CHAPTER VIII

GRADIENTS AND FIELDS: DETERMINATION, DIFFERENTIATION, AND DEDIFFERENTIATION

GRADIENTS, GRADIENT SYSTEMS, AND DEVELOPMENTAL FIELDS

THE term "physiological gradient" has been applied to spatial patterns in living organisms characterized by a gradual progressive differential in certain expressions of physiological condition. That quantitative metabolic differentials seem to be the most conspicuous features in early stages of many forms and that they are essential factors in development is a justifiable conclusion from the data of chapters ii-vii; but concerning the particular chemical reactions and the substrate and how they differ at different levels, we know little. However, if decrease in concentration or amount of a certain substance or substance-complex occurs in one direction along a gradient, there must be increase in concentration or amount of some other substance or substances unless there is decrease in volume. A substance gradient decreasing from apical to basal pole in an egg, for example, must be complemented by another substance gradient, different in character in the opposite direction. In many eggs we find two such opposed substance gradients: the active metabolizing cytoplasm decreases basipetally, yolk acropetally; but the resultant in such cases, at least in early development, may be a single activity gradient, decreasing basipetally. In some other eggs we find no direct evidence of substance gradient; but a single activity gradient, also decreasing basipetally, is nevertheless present and unquestionably associated with graded difference in the protoplasmic substrate. The presence of an activity gradient, as determined or indicated by methods at present available, gives no definite information concerning quantitative or qualitative character of differences in the substrate. Development as a continuing series of changes is an expression of the dynamics of living protoplasms, and very commonly its pattern in early stages appears to be represented wholly or largely by the gradient system present. Specific or qualitative material regional differences are apparently not necessarily concerned in the earliest stages of developmental patterns, though there is no question

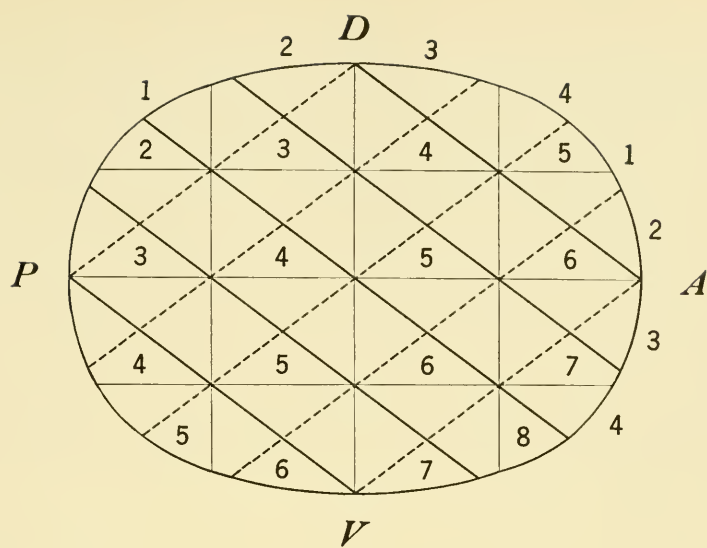
as to their significance in later stages. It is by no means necessary to assume, nor is it probable, that the component reactions are the same in different gradients even in the same individual. On the other hand, it is probable that specific differences begin to appear almost at once in a gradient primarily quantitative; but the important point, so far as early developmental pattern and order are concerned, is that quantitative gradient factors appear to be primary and specific regional differences to develop gradually. The graded differentials in rate of development, oxidation-reduction, electric potential, susceptibility to toxic action, etc., characteristic of these gradients give, of course, only general and partial information concerning them; but since developmental order and pattern show very definite relations to the gradient pattern of which these graded differentials are partial expressions, it appears, beyond question, that the physiological gradients are operative and effective factors in development of axiate order and pattern. The metabolisms of different gradients in the same individual may differ widely, but the gradient pattern is apparently as essential to axiate development as the kind of reaction in it.

As will appear in following chapters, gradients in living protoplasms can be determined experimentally by various environmental factors and under natural conditions are often determined by local activation in relation to factors in the organismic environment of the part concerned. Presence of differentiation is not necessary for their initiation. All that is necessary is localization of an activated region in some way; from this the activation spreads, irradiates, and is transmitted with a decrement in intensity. The activated region corresponds to a region of excitation, and the resulting gradient to excitation transmitted with a decrement. If the activated region persists long enough, it may determine a persistent gradient, which in turn determines axiate development. Whatever the factor or factors initiating activation or a gradient, specific constitution of the protoplasm in which it appears and the physiological condition, as determined by various factors, are, of course, the chief factors in determining the character of the gradient, steepness of decrement and effective length, as well as the kind of physiological activity characterizing it and the kind of development resulting from it. As in nervous excitation and transmission, the activating factor initiates and the protoplasmic mechanism determines character of effect.

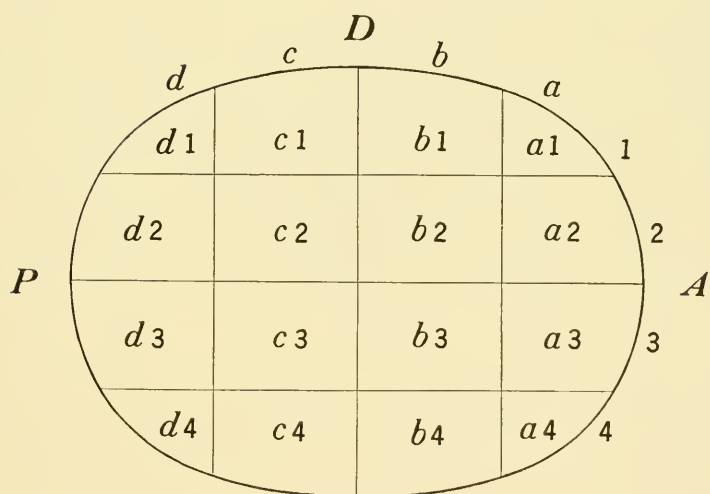
A gradient in a certain direction appears to constitute a physiological basis for definite order and sequence in development in that direction, and a gradient system seems to serve as a sort of a physiological co-ordinate

system with reference to which axiate pattern develops. The question whether and how specific differences can arise at different levels of a gradient is considered in another connection (p. 297); but, assuming for the moment that this is possible, the conception of a gradient system as a physiological co-ordinate system requires some further consideration. A radial gradient system, such as that of many buds, results in a radial developmental pattern which becomes polar in consequence of differential growth (p. 16); and a longitudinal system, like that of a *Corymorpha* piece, results in a longitudinal developmental pattern, within which particular organs or parts arise at particular levels and where scale of organization can be increased or decreased by altering height and length of the gradient (pp. 38, 344-57). But a question at once arises: Can two or more gradients or gradient systems in two or more different directions in the same protoplasm constitute a co-ordinate system which specifically determines each region? In other words, can such a system determine the developmental pattern of organisms with ventrodorsality or dorsiventrality and often asymmetry? If the two or more gradients are of the same kind and intensity, the chief axis of the pattern determined will be in the direction of the resultant of the two. In the accompanying diagram (Fig. 107, *A*) anterior, posterior, dorsal, and ventral are indicated by *A*, *P*, *D*, and *V*. The anteroposterior gradient is indicated by the numerals 4-1 above; the ventrodorsal gradient, by the numerals on the right. The numerals of the different areas are the sums of those indicating respective levels of the two gradients; for the sake of simplicity the two gradients are assumed to be additive in effect. The diagram shows that the chief pattern differences are in the direction of the heavy lines oblique to both gradients. The broken oblique lines indicate directions along which pattern is the same. This is not the sort of pattern usually found in organisms with a longitudinal and ventrodorsal axis.

In Figure 107, *B*, the anteroposterior gradient is assumed to be operative and to have induced some degree of specific differences, *a-d*, at different levels before the ventrodorsal gradient becomes markedly effective, its effectiveness being assumed to increase gradually. In this case each area is in different physiological condition and is defined by its relation to the two gradients, and the pattern has an anteroposterior and ventrodorsal axis. If the ventrodorsal gradient is primarily quantitative, its effect on the specifically different regions *a-d* may differ in character because of their differences and may determine further alteration. Figure 107, *B*, serves merely to suggest in a general way how a developmen-



A



B

FIG. 107, A, B.—Diagrams of effects of two gradients in different directions; explanation in text.

tal pattern with anteroposterior and ventrodorsal axes—or, if the secondary gradient were in the opposite direction, a dorsiventral axis—may arise from a pattern consisting of two gradients at right angles, one becoming operative or being much more effective before the other. As a matter of fact, the ventrodorsal or dorsiventral gradient usually becomes directly evident by the methods at present available, at a later stage of embryonic development than the anteroposterior gradient, although certain experimental data indicate that something constituting a basis for it may be present in the unfertilized egg but with little or no visible effect on early developmental stages. As the diagram is drawn, with the high end of the secondary gradient becoming the ventral region, it might be regarded as representing a plane projection of early planarian or annelid pattern. With the high region dorsal, it might perhaps be regarded as resembling the pattern determined in the amphibian limb bud by its relations with the pattern of the body.

In recent years the field concept has been applied to various developmental phenomena; but before the word "field" came into use in developmental physiology, we find what is essentially the field concept stated in other terms. For example, Spemann (1912a) suggested that early embryonic potency to develop an organ such as the lens of the amphibian eye involves an area analogous to a diffraction circle, the degree of determination being highest in the center and decreasing peripherally. Harrison (1918) says with reference to amphibian limb development:

The limb rudiment may be thus regarded, not as a definite circumscribed area like a stone in a mosaic, but as a center of differentiation in which the intensity of the process diminishes as the distance from the center increases until it passes away into an indifferent region. Many other systems, such as the nose, ear, hypophysis, gills, seem to have the same indefinite boundaries which may even overlap each other.

Harrison is speaking here not merely of the limb bud itself, the locus of actual development of the limb or other organ, but of the whole area about this locus, which is found by experiment to be more or less capable of giving rise to the organ. The concept of the developmental or morphogenetic field has been further developed and applied by various authors independently of, or in relation to, the gradient concept; and, as is usual in such cases, reference of certain developmental activities or results to a field seems sometimes to be regarded as advancing our knowledge of developmental physiology.¹ Without further analysis the field concept,

¹ Spemann, 1921; Gurwitsch, 1922, 1923, 1927; Weiss, 1924, 1926, 1928, 1939; Guyénot et Schotté, 1926a; Guyénot, 1927a, b; De Beer, 1928; von Bertalanffy, 1928; Guyénot et Ponse, 1930; Huxley, 1932; Waddington and Schmidt, 1933; Huxley and De Beer, 1934; Dalcq, 1938.

as applied to development, has only a formal value. Identification of a hydranth field, a limb field, an eye field, or, in general, reference of certain developmental phenomena to a field indicates merely presence and a certain order of capacities or potencies for these phenomena in a certain region but gives us no information concerning the physiological character of the order or the potencies, or the conditions determining realization of potencies in a particular part of the field. In other words, reference to a field merely states experimental data in terms of an unknown, of a concept without definite content, and the field often becomes little more than a verbalistic refuge.

The developmental field concept implies an ordering or controlling factor or factors of some sort. Evidently, however, many developmental fields include differences in actual orders and patterns. For example, in the case of the amphibian limb the area capable, or becoming capable under experimental conditions, of developing a limb is considerably more extensive than the field of actual limb development in any particular case, and the same is true for various other organs and organ systems. Obviously, the potency field and the field of actual differentiation differ in some way and must be distinguished. The individuation field of Waddington and Schmidt (1933) does not throw any light on individuation; and, as the authors apply it to vertebrate development, it seems to suggest that individuation results from action of the chorda-mesoderm as inductor, but actually individuation is present before induction and the chorda-mesoderm is itself a part of the individual.

If the physiological gradients are operative factors in development, the question of the relation of gradients and dominance to developmental fields is important. According to Huxley and De Beer (1934, p. 274), it is found that the original control of differentiation in all cases appears to be exerted in relation to what may be called a biological or morphogenetic field. Within these fields various processes concerned with morphogenesis appear to be quantitatively graded so that the most suitable name for them is field-gradient systems or simply gradient-fields.

This statement raises several questions, for it seems to imply that the field is the factor determining differentiation; yet the field is regarded as a gradient field—in other words, as a gradient system. What is the field, as distinguished from the gradient or gradient system in it? Does the field determine the gradients, or do gradients constitute the field? How does the field determine or control differentiation? What part does dominance play in the field?

The experimental evidence concerning physiological gradients seems to indicate that developmental fields in their simplest, most general forms are gradient systems, that is, the field is constituted by the gradient or gradients present; the gradients are the vectors of the field and determine its extent and the orderly relations within it. Such a field may originate in a region of localized activity which determines a gradient or gradients. In the adventitious bud field of a plant (see Figs. 1-4) the primary region of activity is the physiological center of the field and approximates, in this case, the geometric center; and the gradient system or field is at first radial and becomes axiate and polarized in consequence of differential growth. Such a field may be larger than the actual bud which develops from its higher gradient levels. The earlier stages of these adventitious buds of plants and many buds in animals, whether they develop into complete individuals or parts, such as tentacles, appendages, etc., may be regarded as examples of simple developmental fields. The extent of the region of activation, that is, the extent of the radial gradient system, may or may not be greater than the field of actual differentiation. These are evidently gradient fields, and the field is the gradient system.

Realization of field potencies in actual development is localized in the high region of the gradient or gradient system constituting the field simply because this region is the most intensely active. Experimental activation of some other region of the field may bring about development there, provided it is not inhibited by the dominance of the physiological center. In pieces of *Corymorpha* stem activation of the cells adjoining the level of section determines a gradient or a system of parallel gradients extending for a certain distance (see Fig. 31). This is a reconstitution field; and within it parts of the new hydranth are localized, and alteration and elongation of the stem occurs, so far as length of piece and scale of organization, as determined by intensity of activation and length and slope of the resulting gradient, permit. A similar reconstitution field arises following section in pieces of planarians, nemerteans, annelids, etc., in relation to the activated region which becomes a head. These fields are axiate and polar from the beginning. The dominant region is the high end of the gradient system and is an inductor in the sense that it is the primary factor in determining the gradient system, and consequently the levels at which particular parts shall develop. Such fields as these are fields of actual development, differentiation fields, and represent activated regions of the potency field, which in *Corymorpha* is the whole

stem. During the course of reconstitution in *Corymorpha* new fields—for example, the tentacle fields—arise at certain levels of the gradient system. These also appear to be primarily gradient systems like those of buds; but since they determine particular organs rather than complete individuals, the gradient activities in them doubtless differ in some way from those in the original reconstitution field in which they arise. The appearance of such secondary fields within the primary field indicates that the latter becomes, sooner or later, something more than a simple quantitative gradient system. Apparently specific differences of some sort appear at certain levels, and some of these determine local activation with formation of organ fields at those levels. At present it is difficult to describe the reconstitution of *Corymorpha* in any other terms than these. The gradients arise as the earliest distinguishable evidences of development and can be made visible in the living animals. They may be determined at any level and at either end of a stem piece by section, by a graft from any stem-level, or by a lacerated wound; and their length may be altered and controlled experimentally by environmental factors with corresponding alteration of scale of organization (see chap. x). As already pointed out, such forms of development come nearer providing a starting-point for physiological analysis of development in general than does embryonic development from an egg already more or less highly differentiated beyond the earlier stages of pattern.

Symmetry or asymmetry of bud fields and organ fields arising in relation to pre-existing gradients in the body from which they develop may be determined or influenced by these gradients. The fields of origin of new cilia and cirri in the division and reconstitution of various ciliate protozoa are evidently related in a definite way to the pattern of the original individual;² and some evidence concerning that pattern is given by the ectoplasmic gradient in rate of dye reduction and susceptibility in these forms. The “dorsiventral” or bilateral pattern of tentacle development in the bud of *Pelmatohydra* is apparently determined by the gradient and dominance in the parent body (Rulon and Child, 1937). The anteroposterior and dorsiventral pattern of the amphibian limb arises in definite relation to anteroposterior and dorsiventral pattern of the body.

The question of the relation of metabolism to developmental pattern, and particularly to the developmental field as this is conceived by some,

² See, e.g., Dembowska, 1925, 1926, and citations.

is of fundamental importance. The following discussion of this question is quoted from a recent paper.

Some biologists apparently believe that metabolism is not a fundamental factor in development. For example, Shearer [1930, p. 266] says: "morphological organization has nothing to do with metabolism." Parker [1929, p. 424], in criticizing the gradient concept, makes the statement: "the metabolic activity of the organism is not a true formative process, but the result of such a process." Spemann [1939, pp. 321 ff.] seems to hold somewhat the same opinion; his discussion of gradients involves the mistaken assumption that, according to the gradient theory, there must be only quantitative metabolic differences in the amphibian egg at the beginning of embryonic development. In his recent book, Weiss [1939, pp. 373-83] seems to hold that specifically different capacities for organization exist in different regions independently of metabolism. Granting that these capacities are present in many eggs, how did they arise except through metabolism? And even if they are present in eggs, are they present in the early stages of buds, of pieces of *Tubularia* or *Corymorpha* stem or in aggregates of dissociated *Corymorpha* cells? In isolated pieces of the planarian body, of various annelids and of some ascidians development of a particular part has no definite or constant relation to a particular region of the individual or to a pre-existing organization, but a new organization originates. Does it originate independently of metabolism? If we inhibit metabolism it does not appear and in many cases it is possible to initiate its development by purely quantitative external differentials which in one way or another determine gradients involving metabolism. It has been pointed out repeatedly that, even if these gradients are primarily quantitative, they probably do not remain so for any great length of time, though quantitative factors may continue to exist and be effective, even in many adult animals. How do the regional organizing capacities and the presumably specifically different metabolisms of hydranth, stem and holdfast region in a reconstituting piece of *Corymorpha* originate if not through metabolism? Since any level of a new individual may develop from any level of the stem these capacities are certainly not localized preceding isolation of a piece. . . . The arguments of Spemann and Weiss are based on the egg and embryonic development rather than on development in general. . . . Like many other embryologists they maintain that organization or specific capacity for it is present, but do not tell us how it originates. If organization consists in localized presence of specific substances how can these substances originate and be localized except in the final analysis through metabolism? Probably no one now believes that they are all present in the primitive germ cell. It is difficult to believe, for example, that localized formation of specific substances in the dorsal inductor region of the amphibian egg and embryo can take place independently of metabolism. Without metabolism ovarian development of the oöcyte and embryonic development cease. Even the differentiation of various organs and tissues does not persist if their metabolism is decreased below certain levels. How are electric and other physiological regional differences established and maintained except through metabolism? What is the possible nature of formative processes assumed to be independent of metabolism? In short, is there any more fundamental characteristic of living protoplasms than metabolism? At present evidence of any such characteristic seems to be wholly lacking. It may be argued that structure of some sort is more fundamental, but structure without metabolism is not living protoplasm

and accomplishes nothing that can be regarded as organismic development. Even if the organization of the egg originates in the orientation of elongate dipolar and symmetrical or asymmetrical protein molecules, as Harrison [1937] and others have suggested, the question at once arises: to what do the molecules orient? Metabolism is going on continuously in the ovarian oöcyte and in the parent organism to which it is attached. Can the orientation possibly occur without relation to this metabolism? In reconstitution of a piece of *Corymorpha* or of a planarian the new organization follows certain changes in metabolism in the region concerned and without them does not develop. Doubtless changes in structure are also involved and affect metabolism, but metabolism is the active and effective factor. At present there is no evidence that the changes in metabolism characteristic of the new organization result from orientation of protein molecules in all the thousands of cells concerned. The polarities of the individual cells of the hydroid and planarian are apparently determined by local surface-interior differences without relation to the axiate pattern of the whole, but these cells become parts of the axiate pattern of the new individual and still retain their original polarities. If their molecules are, or become oriented, are they oriented with respect to the local surface-interior factors or to the axiate pattern of the whole individual? If they are locally oriented then the new pattern is independent of them. If the new pattern is determined by their reorientation, how are their original polarities maintained?

Weiss, in his book [1939], has much to say of the field concept, of field energy, of strong and weak fields and of decrease in field energy from a center, but he does not tell us what a field is or may be as an active and effective factor in development, nor does he say what makes a field strong or weak or what the source and character of its energy may be. What is the basis for decrease of field energy from a center to periphery of the field? New fields originate in the course of development: how do they arise? Is their origin independent of metabolism? Is there some other source of energy in a field than metabolism? Without information, or at least hypothesis, concerning these points the field concept remains almost mystical in character.

That organization with localized specific differences of substance and metabolism does originate in some way and that dynamic, not merely structural factors, are essential to its origin appears beyond question; that regional specificities originate and increase during development is indicated by many lines of evidence. If metabolism is merely incidental to, or a result of these changes, it would seem that we must again postulate a specific vital energy: Driesch's entelechy will scarcely serve our purpose, for that was conceived, not as a source of energy, but rather as controlling energy transformation, and that in living protoplasm is in the final analysis, controlling metabolism.

The concept of physiological gradients in terms of dynamic factors effective in bringing about development, rather than of purely structural factors, does not in any way conflict with the concept of organization in terms of specific, regionally localized material differences. It merely maintains on the basis of many lines of evidence that such organization is not the primary pattern of development, but the result of metabolic activity in a primarily quantitative pattern from which the regional specificities gradually developed. In short, this concept is an attempt to look beyond organization already present and to throw some light on the problem of its origin and development [Child, 1940, "Lithium and echinoderm exogastrulation," *Physiol. Zool.*, 13].

The many examples of embryonic developmental fields given by Huxley and De Beer (1934) and by Weiss (1939) make it unnecessary to do more than call attention to a few points of interest here.

THE EYE FIELD OF AMPHIBIAN AND CHICK

His earlier experiments led Spemann to believe that the optic primordia, even those of cyclopian eyes, are determined and localized in the neural plate as a mosaic of independently developing parts.³ Later experiment showed the incorrectness of this view (Spemann und Bautzmann, 1927). The optic primordium was regarded by Stockard (1913 and earlier papers) as first median, spreading laterally and giving rise to two growth centers. According to this view, cyclopia in the amphibian and probably in other vertebrates is fundamentally different from cyclopia in a planarian and from the approximation to, and development in, the median plane of the anal arms of the sea-urchin larva with differential inhibition. There are no grounds for believing that the primordium of the planarian eyespots is originally median, and the primordium of the sea-urchin arms certainly is not. The starfish coeloms become median and single with differential inhibition (p. 219), but no reason appears for postulating an originally median primordium. Moreover, the suckers of the anuran head and the olfactory pits show the same graded approximation to the median plane, single median development, and, with extreme inhibition, absence. Here, also, there is no ground for postulating an originally median primordium.

More recent investigation shows the existence in *Amblystoma* embryos of an eye field including anterior, median, and lateral regions of the neural plate back to a certain level.⁴ Pieces from different parts of this region transplanted to the belly wall give rise to eyes. According to Adelmann in transplantation of pieces without mesentodermal substrate eye development is much more frequent in pieces from the median region (70.8 per cent of transplants) than in lateral pieces (11.1 per cent), that is, eye potency is apparently much higher in the median than in the lateral regions of the field. In transplants with the underlying tissue which induced development of the neural plate 72.6 per cent of the median and 54.4 per cent of lateral pieces develop eyes; that is, the underlying mesentoderm increases eye frequency in lateral but not in median pieces. Moreover, of the median transplants with substrate, 70.8 per cent give

³ Spemann, 1901a, 1903a, 1904, 1912a, b; see also Fischel, 1921, and H. Petersen, 1924.

⁴ Adelmann, 1929a, b, 1930, 1934, with references to earlier work; also Leplat, 1919; Man-
chot, 1929.

rise to two eyes. Also, removal of the mesoderm underlying the anterior part of the neural plate soon after it attains that position results in a high frequency of cyclopia or median approximation of eyes (Mangold, 1931a, p. 365). Adelmann concludes that eye potency is higher in the median region than laterally and that the underlying inductor determines bilateral eye development. The following suggestion perhaps serves to bring vertebrate cyclopia and related modifications more nearly into line with similar modifications in other forms. The inducing action of the underlying mesentoderm unquestionably brings about a change in physiological condition in the ectoderm involving activation, whatever its other effects may be. The mediolateral decrease in inducing capacity of the inductor tissue (p. 459) makes it probable that the change in condition of ectoderm resulting from induction is more rapid and greater in the median region than laterally. In the course of this change the median region passes through a stage of physiological level which represents capacity for eye formation; and since it attains this condition earlier than lateral regions, it may, when physiologically isolated by transplantation at certain stages, give a higher frequency of eye development than lateral ectoderm. Under natural conditions, however, the median region is prevented, by its relations with other parts, from developing an eye or eyes at the stage when capacity for such development is present; instead it undergoes further change in condition, probably with further activation, and finally becomes part of the brain floor, while lateral regions finally attain the condition representing full capacity for eye development. In other words, what develops under natural conditions represents, in general, the full or final effect of induction; the eye potency of the median region represents merely a temporary condition intermediate between the condition preceding induction and the final condition. Eyes are normally bilateral because the final condition constitutes the action system initiating eye development in lateral regions at a certain distance from the median plane and at a certain level of the anteroposterior axis. When the underlying tissue is present in the transplant, the median region may be activated above the level determining eye development; consequently, two eyes, bilaterally localized, develop, the distance from the median plane varying with conditions in the individual transplant. In similar transplants without underlying inductor tissue the level determining eye development is usually present only in the median region, and a single eye is formed. With differential inhibition by toxic agents optic primordia are localized nearer or in the median plane because the more lateral regions never at-

tain the eye-level; with increasing inhibition this level is localized progressively nearer the median plane or becomes median because all lateral regions fail to attain the eye-level. Apparently, however, approximation of eyes and cyclopia cannot be regarded as resulting entirely from differential inhibition of the inductor; inhibition of ectoderm seems also to be concerned. Even the maximum inducing or activating action may not bring any but the median part of the inhibited ectoderm up to the eye-level. The occurrence in fishes of cyclopia with normal brain indicates inhibition largely or wholly ectodermal.

This suggested interpretation not only avoids the assumption of specifically different actions of closely adjoining median and lateral regions of the mesentoderm but also accounts for the change in localization of eye capacity or potency in the course of development and in differential inhibition. According to it, eye potency and optic primordia are not the same: potency may be present in an extensive area, though not necessarily at the same developmental stage in all parts of the area, but optic primordia originate where eyes actually develop. The eye field appears to be primarily a gradient system resulting in large part or wholly from induction in the ectoderm and subject to experimental alteration with altered localization of optic primordia.

The eye field of the chick embryo at certain stages, as indicated by chorio-allantoic grafts of different regions of the blastoderm, is also a bilateral field including median and lateral regions.⁵ At these stages eye development or differentiation of eye tissue occurs in both median and lateral pieces from a certain blastodermal level, but more frequently and with more advanced differentiation in median pieces.⁵ A slight asymmetry of the field is indicated by a larger amount and more advanced differentiation of eye tissue from left than from right lateral grafts. The presence of a mediolateral gradient of susceptibility and rate of dye reduction in the chick blastoderm has been shown (pp. 159-62). Except as regards the asymmetry, the interpretation suggested above applies here also. If it is correct, the mediolateral extent of the eye field in the amphibian and bird, the higher eye potency in transplants of median regions at certain stages, and the approximation of eyes and cyclopia under inhibiting conditions are all expressions of a mediolateral pattern, primarily a quantitative gradient pattern rather than regionally specific; and approximation of eyes and cyclopia in vertebrates do not differ in principle

⁵ Clarke, 1936; Rawles, 1936. See also Figs. 167, 170, pp. 531, 534.

from approximation to the median plane of other bilateral organs in vertebrates and invertebrates under inhibiting conditions.

One other point requires notice in connection with these transplantation experiments on parts of the eye field. It is by no means certain that isolation and transplantation of a piece of the embryo is possible without any alteration of physiological condition of the piece. In the light of experiments with hydroids and planarians it appears possible that a temporary stimulation may follow isolation and perhaps be followed in turn by a depression. If the pattern is a gradient pattern, such changes will certainly play a part in determining what develops from the transplant. There may be differences in susceptibility to the altered environment in pieces from different regions of the field, and these may influence the developmental result. In short, potency for eye development or for any other development may depend as much on the environment of an isolated or transplanted piece and the operative effects on it as on the condition of the region concerned before operative procedure.

THE AMPHIBIAN LIMB FIELD

In consequence of the great amount and variety of experiment more is perhaps known concerning fields of organ systems and organs in amphibian development than in other forms. Among these the amphibian limb field has received much attention and serves as an excellent example. The limb arises laterally on the body; and the potency field, the region in which limb development can occur, is in earlier stages more extensive than the region of actual development in any particular case. Its physiological center, where the potency is highest, and where the limb normally develops, is apparently nearer the anterior than the posterior border, that is, in the higher levels of the portion of the anteroposterior gradient included within the field. In the primitive limb disk the anterodorsal quadrant is apparently to be regarded as the dominant region (Swett, 1923), suggesting that perhaps the dorsiventral gradient of the body may also be concerned in its localization. The frequent duplication or triplication of limbs in connection with transplantation of limb buds and regeneration of limbs, evidently a result of the appearance of more than one physiological center, is discussed in another connection (pp. 390-95). As others have pointed out, the limb field is not established all at once; but certain of its characteristics appear at one stage, others at another. It is not a static pattern but a product of continuous progressive change. It is at least a pertinent question whether anything other than a metabolic pattern with

progressively increasing regional specificity can account for the establishment and development of this field. In *Amblystoma* the pattern of the limb bud is determined in the anteroposterior direction of the body earlier than the pattern in the dorsiventral direction; and both of these before the mediolateral, that is, the longitudinal or polar axis of the limb.⁶ Anteroposterior and dorsiventral axes are apparently determined about the same time in anura and in *Triton*, but experiment on these forms is less extensive than on *Amblystoma*.⁷ Determination evidently follows much the same course in the regenerating as in the original limb bud. Apparently, the anteroposterior and the dorsiventral gradient pattern of the body are imposed on the limb primordium. In *Amblystoma* the dorsiventral pattern is less effective and becomes fixed in the limb primordium later than the anteroposterior. The longitudinal axis of the limb, however, is apparently determined, as in other buds that become axiate, by the gradient system in the bud itself. The radial decrease in developmental activity about the physiological center becomes a longitudinal gradient as outgrowth occurs.

That there is a real effective dominance in the limb field is suggested by the fact that a limb region removed from its original position and implanted on the flank of the same or another animal is usually inhibited or resorbed if within a certain distance of the limb regenerating from the original site or of a normal developing limb, but develops if at a greater distance (Detwiler, 1918; Hellmich, 1930). It might perhaps be expected that the transplanted limb region, since it represents the region of highest potency in the field, would dominate and inhibit regeneration from the original site. Its failure to do this may result from depression brought about by removal and implantation in a new environment, or the activation in the original site following its removal may be sufficient to inhibit it. Even though polarity of the limb in regeneration is not determined by the proximal stump or by the potency field of the limb, the limb field plays a part in determining the character of distal regenerating or transplanted parts. The regenerating outgrowth on the stump of an amputated fore leg transplanted in an early stage to the stump of an amputated hind leg may develop as hind leg, or that from a hind leg as fore leg when transplanted to a fore-leg stump (Milojevic, 1924). It may be suggested that the determining factor in this case is the difference in the asymmetry

⁶ Harrison, 1921a, b, 1925a; Swett, 1926, 1927, 1928a, b, c, 1932, 1937a, b, 1938a, b, c, 1939; Swett, 1936, experiments on hind limb. See also pp. 390-95.

⁷ Gräper, 1922a, b, 1923, 1924, 1925, 1926a, b; Brandt, 1924a, b, 1925; Milojevic, 1924.

patterns of fore-leg and hind-leg stumps, for this asymmetry pattern presents a differential or gradient pattern at the cut surface of the stump which may be imposed on the regenerating tissue and perhaps also determines the asymmetry pattern of the transplant. But the regenerating outgrowth on a limb stump is not limited or definitively determined in its earlier stages as regards development into a limb, for it may develop into a tail-like structure if transplanted to the tail region (Guyénot, 1927; Guyénot et Ponse, 1930). According to Weiss (1927*b*), the regenerating outgrowth of an amputated tail transplanted to a limb stump may develop as limb. In these cases, also, the symmetry pattern of the tail or the asymmetry pattern of the limb is probably the factor determining the character of development. However, Liosner and Woronzowa⁸ find a considerable specificity in transplants of muscular tissue from tail to limb, etc.

Implantation of the limb region with more or less rotation in the dorso-ventral plane from normal orientation is sometimes followed by rotation to normal orientation.⁹ This reorientation apparently occurs in relation to the region from which the shoulder girdle develops, not to the body as a whole, and has been regarded as in some way associated with the shoulder girdle. A small implant ($1\frac{1}{2}$ somites) which develops no shoulder girdle and a large implant (5 somites) which develops a complete girdle do not rotate, but an implant of intermediate size ($3\frac{1}{2}$ somites) which develops part of a girdle may rotate. If a $3\frac{1}{2}$ -somite region and a ring of tissue representing the region around it are separated and implanted with independent and different rotation, the limb region rotates to normal orientation with the ring. The question arises whether the girdle as such or the gradient pattern is the factor determining rotation. Undoubtedly, both the asymmetry gradient pattern of the part undergoing rotation and that of the part on which it rotates must have attained a certain degree of development in order that rotation may occur. The fraction of the pattern in the $1\frac{1}{2}$ -somite piece may be insufficient to bring about reaction, and the 5-somite piece may fail to rotate because of physical conditions associated with its size or because it possesses sufficient pattern to alter adjoining regions to some extent instead of rotating in reaction to them.

It appears highly probable, in view of the data, that the gradient system or field about the transplanted or regenerating limb primordium does, or may, influence it and impose pattern on it, or determine postural rotation. The fact that an anteroposterior asymmetry of the limb coincides

⁸ Liosner and Woronzowa, 1937; Liosner, 1938; Woronzowa, 1938.

⁹ Harrison, 1921*a*; Nicholas, 1924, 1925, 1926.

approximately in direction with the anteroposterior axis of the body and is apparently the expression in the localized limb region of that pattern is perhaps significant as indicating that polarity and asymmetry are not fundamentally different in character; something constituting polarity in the body as a whole determines an anteroposterior asymmetry in the limb; similarly, something constituting dorsiventrality and bilaterality in the body determines a dorsiventral asymmetry in the limb. It seems difficult to account for facts such as these except in terms of gradient pattern. Apparently, work is done in the postural rotation of an implant, and this implies a dynamic factor of some sort.

Within the potency field of the limb development of a limb can be induced not only by implantation of part of a limb bud but of an otic vesicle, an olfactory placode, brain tissue, eye, or even a piece of celloidin and also by a nerve deflected to a region of the field. The induced limb appears later the farther posterior the position of the inductor and reactivity to an inductor disappears from anterior to posterior regions of the field progressively with advance of development.¹⁰

The inductor, whether implant or nerve, apparently serves in these cases merely as a nonspecific activator, and its action seems to be primarily on the mesenchyme. The developmental result is determined not by the inductor but by the polar gradient or gradients resulting from the localized activation and outgrowth, the character of the field in which the outgrowth takes place, and the asymmetry pattern representing a part of the general body pattern. Either ectoderm or mesenchyme of the axolotl limb bud at certain stages may determine limb development with foreign mesenchyme or ectoderm, according to Filatow (1930a). This may mean that both are activated sufficiently so that either can act as a dominant region.

SOME OTHER FIELDS

Experiment has shown the existence of various other fields in amphibian development—ear, gills, urodele balancer, etc.—and in many of them gradient characteristics appear. Like the limb and eye fields, the potency field of the ear is more extensive than the differentiation field in earlier stages. Otic development induced in other parts of the field than the physiological center is more complete near the normal differentiation field than at greater distances, and the anteroposterior axis is determined earlier than the dorsiventral.¹¹ Implantation of the presumptive ear region

¹⁰ Detwiler, 1918; Balinsky, 1925, 1926, 1927, *a, b*, 1933, 1937*a, b*; Filatow, 1927.

¹¹ Yntema, 1933; Harrison, 1936*a*; Albaum and Nestler, 1937; Hall, 1937, 1939.

of *Amblystoma* in the normal differentiation field of the ear (orthotopic implantation) with various degrees of rotation may result, at certain stages, in reversal of both anteroposterior and dorsiventral axes, or one may become the other. It seems evident that these changes in pattern are imposed on the implant by the pattern about it.

The field of the larval urodele balancer in the ventral head ectoderm is apparently not axiate, and the balancer itself appears to be radially symmetrical. A radial potency gradient decreasing from a physiological center has been demonstrated; ectoderm from this region, but not other ectoderm, transplanted to other head regions at certain stages, will determine development of a balancer with mesoderm from the region of implantation. In later stages just preceding appearance of the balancer bud transplanted ectoderm of this region will determine balancer development in trunk as well as in head regions. Harrison (1925*b*) regards this difference in inducing capacity as indicating increase in specificity, but it seems entirely possible that it may indicate merely a more intense activation of the balancer ectoderm at the stage when outgrowth is about to begin. Development of supernumerary balancers in the field can be induced by implants of various tissues, neural plate cells, foregut cells, even tissue from urodeles which have no balancer (Mangold, 1931*b*) and cells of the anuran neural crest (Raven, 1931, 1933*b*). That the inducing agent is anything more than a nonspecific activator here, as in limb induction, seems improbable.

These examples are sufficient to indicate the character of field phenomena in amphibian development where they have been most studied. That the potency field is primarily a gradient system of a certain kind and that the normal differentiation field represents its high region is indicated by various lines of experimental evidence. The limb field, the eye field, the ear field, appear in the course of development, but their patterns may not be established all at once. The limb field is a region from which limb develops before the pattern of a particular limb is finally fixed, that is, the pattern of the limb may be altered by transplantation to an altered physiological environment. According to data at hand, amphibian potency fields, as areas more extensive than the fields of actual differentiation, undergo progressive restriction in most cases with progress of development and finally disappear completely. The possibility remains, however, that under other experimental conditions potencies might appear which now seem to be absent. The disappearance of a potency field is evidently associated with the progressive differentiation of its different

regions in different directions. In many hydroids and planarians there is no progressive limitation of potency except in the hydranth or the head.

At present it appears that developmental fields are not sharply and definitely bounded in their earlier stages, and it has often been suggested that different fields may overlap so that a certain region may be part of two fields at the same time, but direct proof is lacking. If fields are primarily gradient systems, clearly defined boundaries are not to be expected, and extent of a field may increase or decrease with the activity in it. If such changes occur, certain regions may be parts of different fields at different times. Within the field, as in development in general, stable structure and differentiation appear to be secondary and to result from a pattern primarily nonspecific and labile with a graded metabolic differential. That a field in its most general form is anything more than a quantitative gradient system of a particular kind remains to be proved and in the light of experimental evidence appears improbable.

HARMONIOUS-EQUIPOTENTIAL SYSTEMS

Driesch introduced the concept of harmonious-equipotential systems and in one of his papers characterized them as follows: "Each part can give rise to any part ['jedes Element kann jedes'] and each effect [i.e., developmental result] occurs only once or a definite number of times and in a fixed relation to all other effects" (Driesch, 1899a, pp. 73-74). He regarded the *Tubularia* stem and cleavage stages of the sea urchin as such systems and later discovered what he believed to be other systems of the same sort. The term has since been applied to various developmental systems, among them the amphibian limb field. The existence of these harmonious-equipotential systems was regarded by Driesch as evidence for "autonomy of vital processes," that is, for so-called "vitalism." It is a fact amply confirmed by many investigators that any level of the *Tubularia* stem can develop into any body-level of *Tubularia*. This is also true for *Corymorpha* and many other hydroids and for the postcephalic regions of various planarians, nemerteans, and annelids. However, the various body-levels, even the levels of the *Tubularia* and *Corymorpha* stems, are equipotential only specifically, not quantitatively, at any given moment, that is, any of them can give rise to the various parts of the body but scale of organization and rate of development decrease from distal to proximal or from anterior to posterior levels. That is, individuals developing from different levels are primarily different. The amphibian

limb field is not equipotential at any given moment. Any other area in it than the presumptive limb area must undergo change to become capable of giving rise to a limb, and limbs from some parts of it may be less complete than those from other parts. In short, for every particular development the system, whether *Tubularia* stem or limb field, must become another system; since this is possible, it is equipotential in one sense, and since it has a pattern, or a new one is produced in it, it is harmonious. According to this view, the harmonious-equipotential system appears to be a gradient system in which the different levels have not become so far specifically different that they cannot react to altered conditions with an altered development, that is, a system in which a similar pattern may be differently localized under different conditions. In these terms it is far from constituting proof of the autonomy of vital processes; but if organismic pattern consists primarily in definitely localized specificities, as Driesch assumed, it becomes difficult to see how a part can become a whole without the aid of Driesch's entelechy or some other equally capable metaphysical agent. As a matter of fact, various evidences that inequipotentialities appear in systems assumed to be equipotential are found in Driesch's data; but either they were ignored, or in certain cases it was maintained that they represented results of mere physicochemical conditions without the controlling action of entelechy.

DETERMINATION AND DIFFERENTIATION

In experimental analysis of development the terms "determine," "determination," and "differentiation" are so generally used that some consideration of their usage and the basis for it seems necessary. To determine experimentally a certain developmental result is to provide the conditions necessary for it. We can determine certain differential modifications of pattern by exposure of the entire developing organism to toxic agents (chaps. v-vii). New polarities and symmetries can be determined in various ways (see. chap. xi). In recent years, however, we have come to speak of determination of regions or parts in the course of development. A part not yet visibly differentiated, that is, not yet morphologically characterized, is regarded as determined when its development continues unaltered—at least for some time—after change in, or isolation from, organismic environment. Such a part is said to be capable of self-differentiation. The change in environment may consist in transplantation to another region of the same individual or to other individuals of the same or other species; that is, it may be autoplasmic, homoplasmic, heteroplasmic,

or xenoplastic, or it may consist in isolation in water or some other medium. Different degrees of fixity, stability, or irreversibility of determination are recognized according to the development of the part in different environments. If it shows capacity for self-differentiation in certain environments, not in others, determination is said to be "labile." Often however, conclusions concerning fixity or irreversibility of determination are drawn from a single change of environment. Self-differentiation in the altered environment shows, of course, that determination or segregation (F. R. Lillie, 1927, 1929) has taken place, but that it is fixed or irreversible in all environments does not necessarily follow. It has been pointed out by Harrison (1933) and by Gilchrist (1933) that determination is or may be relative and may be evident in one environment and not in another. Transplantation experiments with urodele fin ectoderm provide an excellent example of the relative character of determination (Twitty, 1939). Many regions or parts found by experimental alteration of environment to be more or less determined are not morphologically distinguishable from other parts at the time of alteration, but their later development shows that differences of some sort must have been present at that time.

The development of the concept of "formative substances" has led many investigators to believe that determination in development results from the presence in the part concerned of a substance or substances different from those in other parts. In the earlier stages of determination formative effects of such substances may not yet have become sufficient to be directly distinguishable. Development of morphological form consists in local differences in growth rate, in cell movement, pressure, tension, turgor, viscosity, etc., and in the metabolic reactions of the protoplasmic system and the character of the substrate; it is the expression of an exceedingly complex action system. That the systems concerned in determination of a hydroid tentacle, a planarian head, or an amphibian limb differ from other parts of the individual—at least after a certain stage of development—is evident; but the tentacle, the head, and the limb result from a definite spatial activity pattern, not merely from presence of a particular substance.

With the progress of experiment it becomes increasingly evident that the term "formative substance" is a misnomer. We find formative patterns with metabolism as an essential factor. The reaction patterns, rather than substance or substances, are the formative agents. A particular substance may determine a certain kind of metabolism, but it is the spatial

pattern of molar magnitude in which metabolism occurs that is determinative and formative. Properly speaking, formative substances do not exist. Even assuming that localization of a particular substance in relation to the polar pattern of a hydroid determines the level or region where a tentacle shall develop, tentacle development results from a particular spatial activity pattern in that region; this is no less true for the amphibian limb field. A chemodifferentiation of a field without an activity pattern cannot determine morphological form. There is, at present, no actual evidence that the character of metabolism in the region where a tentacle or a limb will develop is different in earlier stages from that in other regions. Even in later stages the same structures—skin, muscle, connective tissue, bone—develop in the limb, in the tail, and in various other parts of the amphibian body. The difference between these parts is in the spatial developmental pattern rather than in substance. As regards anteroposterior and dorsiventral axes, the amphibian limb field represents a certain relation to general body pattern, different from that of other parts. In this pattern a new gradient system arises and becomes the longitudinal axis of the limb. The limb as a pattern is specific and undergoes a definite orderly series of changes, with the limb as the result. Different substances are formed in different regions of the pattern, as in other gradient patterns; but again it is the pattern in which the substances are formed, not the substances, which constitutes the limb.

Determination is commonly supposed to become increasingly stable in the course of development, and this is usually considered to indicate increase in specificity or chemodifferentiation, but determination is sometimes apparently merely a matter of gradient-level. A piece of *Corymorpha* stem from a high gradient-level transplanted to a low level may dominate its environment and develop into an apical region, as it would have done if isolated, and may even induce other parts in the host; a similar piece from a low gradient-level, capable of the same development as the other, when transplanted to a high level may be incorporated and develop merely as part of the stem. The one might be regarded as stably determined, the other as labile. The relative character of lability and stability of determination is shown in many lines of experiment. Various data from isolation and transplantation of parts of the sea-urchin embryo indicate stable determination of the apical region as ectoderm, but in the extreme forms of exogastrulae (Fig. 91, *H, I*) and with transplantation of micromeres to the apical pole (pp. 443-44) it is found that the apical region can develop as entoderm.

Transplantation experiments with amphibian material have shown in many cases more or less lability as regards region of the body formed but complete or relatively high stability as regards species or group characteristics of organs formed (pp. 457, 499). On the other hand, certain species and group characteristics of larval form in echinoids and asteroids can be altered to an extreme degree and in different directions by differential inhibition, conditioning, and recovery, that is, by altering the gradient pattern (chap. vi).

Doubtless many cases of determination do represent increase in specificity over the undetermined condition and may be regarded as the beginnings of differentiation, an "invisible differentiation" (Gilchrist, 1937*a, b*). The view that determination is not a gradual development of specificity but a relatively abrupt restriction of potency of a part, and that it is always dichotomous, has been advanced by F. R. Lillie (1927, 1929). So far as determination represents attainment of a certain degree of specificity in a certain region of a gradient pattern, its gradual origin and development seem equally possible, and Lillie's critical period may represent the threshold of attainment of a certain degree of specificity permitting self-differentiation. Lillie regards determination as an independent variable in development; but if it is a resultant of gradient pattern, it is by no means independent.

The concept of differentiation is perhaps the most indefinite and most loosely applied of any concerned with development. In a morphological sense a part of a cell, a cell, or a cell group is commonly regarded as differentiated when it differs visibly in structure from an earlier "embryonic," supposedly undifferentiated condition; sometimes a change of shape has been regarded as differentiation. On the other hand, certain cells, although visibly different in appearance from embryonic cells, are often assumed to be indifferent or undifferentiated because they give rise in reconstitution to parts other than those which they formed in the original individual. Moreover, as noted above, chemodifferentiation is often inferred in parts capable of self-differentiation, though other evidence is lacking. The more advanced stages of histological and organ differentiation are directly and clearly distinguishable in the higher animals, and the morphological differentiation is generally paralleled by specificity in chemical constitution and in character and products of metabolism; but exactly when or how a particular differentiation begins, we do not know. Conclusions as to presence or absence of differentiation are often purely matters of opinion determined by more general opinions concerning de-

velopment. For example, one who believes that, once differentiation is initiated, regression is impossible is likely to ignore appearance, structure, and function of certain cells and to regard them as undifferentiated because they differentiate into new organs in reconstitution. The so-called "formative cells" in certain organisms are examples. Others, who believe that dedifferentiation is possible, are likely to regard such cells as more or less differentiated and as undergoing dedifferentiation in the activation and new development of reconstitution.

It is an interesting question whether differentiation is entirely cytoplasmic or may involve the nucleus. Certainly, the nucleus takes on many different forms and appearances in different cells. It becomes polymorphic, sometimes highly branched, in other cells much condensed, and its staining properties may differ greatly in the same cell at different times. The nucleus of the ovarian oöcyte, for example, usually differs greatly in appearance and staining from that of maturation and later stages. Nuclei of most spermatozoa differ greatly from other nuclei of the same species. Certain blastomeres of *Ascaris* undergo diminution of chromatin in early cleavage stages; and following this change, number of chromosomes is greater, size less, and appearance very different. Moreover, localization of diminution in certain cells in relation to centrifuging and dispermy indicates that regional cytoplasmic differences determine which nuclei undergo this change and which do not (Boveri, 1910b; Hogue, 1910). Whether these and many other nuclear changes appearing in the course of development of gametes and other cells are to be regarded as differentiations is at present largely a matter of opinion. A cytoplasmic environment seems to be necessary for long-continued nuclear life, and the nucleus is certainly not wholly insensitive to change in this environment; that nuclear differentiation may be induced by the cytoplasm in some cells seems probable, and perhaps nuclear self-differentiation is possible.

The question of the basis of differentiation has interested biologists since the study of development began. The Roux-Weismann theory of qualitative nuclear division as the basis of differentiation is now discarded. In the ordinary mitosis each daughter cell is supposed to possess the same genic constitution as its parent. As Morgan once put it (1919, p. 241), "each cell inherits the whole germ plasm." But cells and cell groups become increasingly different from each other in the course of development. How is this possible?

Undoubtedly, there is interaction between nucleus and cytoplasm, and supposedly different genes become activated or in some way come to

play a part in determining the differences in different cells; but unless the genic system is similar to Dreisch's entelechy, differences in gene effect must be based on conditions in the cytoplasm. We can scarcely conceive that substances produced by the genes can arrange themselves in an orderly definite pattern resulting in the regional differentiation characteristic of the individual and species, and there is nothing in nuclear pattern that suggests the spatial pattern of differentiation. An orienting and ordering factor of some sort giving rise to a cytoplasmic pattern independently of the nucleus appears necessary for development. This pattern must be the basis of physiological polarity, symmetry, asymmetry, and of morphological form and differentiation in general. In preceding chapters the presence of physiological gradients involving differences in metabolic rate has been shown to be a feature of developmental pattern and to be definitely related to the course of differentiation, but whether such gradients are the primary factors of pattern or results of a still more fundamental pattern may still be questioned.

An "intimate structure," an orientation of molecules or of colloidal particles crystalline in structure, or a space lattice have been assumed by various authors to be the basis of developmental pattern. As regards such hypothetical structures, it may be noted, first, that there is no evidence of their existence as general characteristics of pattern. Orientation of molecules undoubtedly occurs in relation to interfaces, phase boundaries, etc., and many highly differentiated structures give evidence of orientation of molecules or particles; but there is no evidence that the polarity originating in a hydroid or planarian piece undergoing reconstitution results from orientation of molecules in all the cells involved or from a space lattice extending through the whole. It is difficult to believe that such a structure could persist even in eggs and embryos through all the changes in position and form of cortical as well as other parts of the cytoplasm. Second, if such structure exists, its orientation presumably originated in relation to something. The free pole of the ovarian oöcyte becomes the apical or animal pole of the egg and embryo in many forms. If this polarity consists in a molecular structure, how was that structure oriented in that particular direction? That a metabolic differential is present between free and attached poles of the oöcyte is sufficiently evident in many cases and appears beyond question in others. If such a differential is present, it is highly improbable that a polar molecular orientation could occur independently of it; but, if it is the orienting factor, it, rather than the molecular orientation, is the primary polarity and the postulated molecular

structure appears unnecessary and, if present, is a result, not a cause, of physiological polarity. Third, the question how such a molecular structure can bring about organization and morphological differentiation remains. It is suggested by Harrison (1937) that the polar, symmetrical, or asymmetrical structure of protein molecules brings about the localization of different substances at opposite poles of the egg or in relation to symmetry or asymmetry. Developmental pattern, according to these views, is primarily structural and static. Recent X-ray diffraction photographs of various embryonic tissues of amphibians and the chick have failed to show any evidence of molecular orientation that might constitute a basis for spatial developmental pattern. The authors, however, point out that these negative results do not prove the absence of such orientation (Harrison, Astbury, and Rudall, 1940, "An attempt at X-ray analysis of embryonic processes," *Jour. Exp. Zool.*, 85).

Turning to the gradient concept, the following points are of interest in relation to the problem of differentiation. The physiological gradients are real, not hypothetical; they are activity gradients involving protoplasmic dynamics as well as substrate; when they are altered, the course of differentiation is altered (chaps. ii, v-vii). When they are obliterated, axiate development and differentiation do not occur, even after the inhibiting factors have been removed. New patterns can be initiated by external differentials which affect metabolic rate (see chap. xi). A relation between the gradient pattern and differentiation is evident, but there is still the question whether this pattern provides an adequate basis for differentiation. The differences distinguishable at different gradient-levels in early stages appear to be primarily quantitative; but what is quantitative and what qualitative in an activity gradient in a living protoplasm is perhaps a somewhat academic question. Assuming, however, that a gradient may be primarily quantitative, at least as regards its dynamic characteristics, can specific or qualitative differences originate at different levels of it? Uptake of oxygen, intake and transformation of nutritive material already present, breakdown of certain molecules, synthesis of others, and discharge of CO_2 and other metabolites are factors of the metabolism of living protoplasts. In a region of high rate of metabolism, transformation of nutrition may occur as rapidly as it becomes available, in part perhaps by complete oxidation, in part by partial breakdown, recombination, and synthesis of new molecules. In a region of lower rate concentrations of nutritive material and of oxygen available in relation to rate of transformation are undoubtedly different, and metabolism there may result in

formation of different products. Differences in concentration of electrolytes and their ions must also occur at different gradient-levels. Electric-potential differences are apparently characteristic of gradients. Different rates of metabolism involve differences in enzyme activity. Present knowledge of molecular constitution of proteins and the part which they play in enzymes suggests that many of them may be extremely sensitive to differences such as these and that positions and relations of groups in a molecule may differ at different levels with resulting difference in reactions. The chain of reactions concerned in oxidations is probably not the same with different concentrations of reacting substances. Certain substances tend to concentrate in regions of greater or less surface energy. These and doubtless many other factors may be concerned in originating differentiations at different gradient-levels. Relation and interaction between levels appears also to be an important factor in differentiation. There is no theoretical difficulty as regards origin of specific or qualitative differentiations at different levels of a primarily quantitative gradient. In fact, it is difficult to believe that such a gradient can remain without some differentiations for any considerable time. The progressive increase in specificity of particular parts so generally characteristic of development also suggests that the primary pattern may be without regional specificity. If a gradient extends over more than a single cell, cells along its course represent different levels, and these differences provide a basis for difference in gene action, certainly an essential factor in differentiation. The character of metabolism in a gradient within a single cell is undoubtedly also determined by interaction between nucleus and cytoplasm. According to this conception, determination and differentiation of parts are earlier and later stages of a continuous series of changes: the primary pattern which initiates these changes and determines their orderly relation along a physiological axis is a gradient in which differences in rate of metabolism constitute the effective factor.

The assumption that axiate developmental pattern consists primarily of a static structure or of localized specific substances seems to involve confusion of the dynamic and material aspects of living protoplasts and of development. Developmental pattern appears primarily as an activity pattern with localization of specific substances and morphological structure as a result. Investigation of organization and development has been largely in the hands of those with morphological, rather than physiological, training; and viewpoint and theories of development have usually been based on embryonic development alone. This situation is perhaps

in part responsible for the character of much of developmental theory. But the primary developmental pattern is no longer present in most animal eggs at the beginning of embryonic development; more or less structural organization is usually already present. Many buds and reconstitutions of individuals from isolated pieces or cell aggregates involve origin of a new developmental pattern, which is accidental as regards both time and place, so far as the original individual is concerned. That these forms of development are not merely the further expressions of organizations already present in consequence of the particular past history of a single cell, as embryonic development is, appears evident. How completely independent of pre-existing organization they may be will appear more clearly in later chapters. It is to these forms of development, rather than to the egg and embryo, that we must look for the beginnings of developmental pattern and the physiological bases of determination and differentiation of parts.

DEDIFFERENTIATION

Differences of opinion concerning the possibility of dedifferentiation are, of course, associated with the question whether certain cells are or are not differentiated. If cells which take part in the reconstitutive development of other organs than those of which they were originally parts are differentiated, they presumably undergo more or less dedifferentiation. Those who regard them as differentiated usually conclude that they dedifferentiate and redifferentiate in reaction to the altered environment. Others apparently conclude that, because they reconstitute other organs, they must be undifferentiated. The question of reversibility or regressibility of development in general is, of course, essentially the same question and open to this difference of opinion.

Weismannian theory necessarily assumed that reversal or regression of differentiation is impossible, that a cell which has once begun to differentiate can never return. Only the germ plasma does not differentiate. As a matter of fact, however, the egg and the spermatozoon of most animals appear to be very highly differentiated cells, perhaps the most highly differentiated cells of the individual. They appear to have approached or attained the limit of possible differentiation in two different directions. The yolk is both a chemical and a structural differentiation; the motor apparatus of spermatozoa can scarcely be regarded as undifferentiated cytoplasm; and the sperm nucleus is certainly in very different condition from an embryonic nucleus. When fully differentiated as gametes, both

egg and sperm are near death and, except for parthenogenetic eggs, inevitably die unless fertilization occurs. Naturally parthenogenetic eggs show, in general, less extreme morphological differentiation than those requiring fertilization (Child, 1915*b*, chap. xiii). The sperm cytoplasm may not take part in the changes following fertilization; but when embryonic development begins, the egg begins to lose its egg characteristics, and sooner or later the resulting cells attain what is commonly called "embryonic condition." The pattern of organization in the egg, however, becomes the basis of embryonic pattern. The earlier stages of embryonic development appear to involve a considerable dedifferentiation from the egg condition with progress of a new differentiation within the general egg pattern. If these changes are not dedifferentiation, there is probably no dedifferentiation in any cell.

That developmental determination can often be experimentally altered has been abundantly demonstrated. If such labile determination represents an early stage of differentiation, its alteration must be a dedifferentiation. Under certain conditions synthesis and accumulation, and under other conditions decomposition and loss of a substance or substances, take place in cells. These changes appear to be differentiation and dedifferentiation.

The assumption that certain cells are undifferentiated because they give rise to new organs in agamic and reconstitucional development is open to criticism on two grounds. First, it ignores the changes in structure and behavior usually evident in these cells; second, it involves the assumption that these cells have been insensitive to their physiological environment during the preceding development but suddenly become sensitive to conditions resulting in budding, fission, or reconstitution. The epidermal cells of the begonia leaf are very different from embryonic cells in structure and behavior. They have formed cellulose membranes and a large vacuole and under the usual conditions would never divide again; but under the conditions initiating bud formation their cytoplasm changes in structure and staining properties, they begin to divide and grow, cellulose membranes disappear, and a gradient system with vegetative tip in its high region develops and gives rise to all the structures of the plant axis (pp. 17-19). Fission and reconstitution in ciliate protozoa apparently involve extensive ectoplasmic dedifferentiation and redifferentiation. Old cilia and cirri "melt down" into the ectoplasm, and new ones develop from other regions.¹² Either the ectoplasmic structures of these animals

¹² E.g., Dembowska, 1925, 1926; Lund, 1917. Many other papers give similar data.

do not represent differentiation, or dedifferentiation and redifferentiation occur. Huxley and De Beer (1923) have described what they regard as dedifferentiation in the hydroid *Obelia* under inhibiting conditions. The cells of stem pieces of *Corymorpha* seem to become less differentiated under conditions that obliterate polarity and lead to development of new axes from lateral regions. Cell changes in sea-urchin larvae, regarded as dedifferentiation but resulting in degeneration and death rather than redifferentiation, have also been described by Huxley (1922). The parenchyma cells of planarians and nemerteans are very different in appearance from embryonic cells of the species, but in reaction to section they undergo change in structure and behavior and develop into various organs. These cells, or some of them, are assumed by some to be undifferentiated "formative cells," apparently because of the part they play in regeneration, while others maintain that dedifferentiation takes place.¹³ Regeneration of the annelid central nervous system from ectoderm involves active proliferation and loss of epithelial character. In the intact animal these cells secrete cuticle and have a certain structure and form different from that of nerve cells. According to Nuzum and Rand (1924), cells of the pharyngeal epithelium can also give rise to nervous tissue. Pharyngeal and nerve cells certainly appear to be differentiated in different directions. Cells usually regarded as of mesodermal origin, the neoblasts, play a considerable part in regeneration of other annelid organs and are regarded by some authors as embryonic or undifferentiated cells.¹⁴ Faulkner (1932) maintains that these cells do not come from the coelomic wall, as described by others, but from outside it, that is, from the blastocoel, and that they give rise to germ cells as well as regeneration cells. Sayles (1927) and Weitzmann (1927) regard them as mesodermal cells that have resumed active proliferation. They certainly undergo change in appearance and behavior after activation following section and develop into other organs than the coelomic wall. R. G. Stone also regards them as mesodermal in origin and finds that X-rays inhibit their activation but also inhibit activation of other cells in *Tubifex* and consequently inhibit regeneration; but this is far from proving that neoblasts are specifically "formative

¹³ Formative cells: Curtis and Schulze, 1924; Curtis and Hickman, 1926; Curtis, 1928; Collings, 1932; Coe, 1934a, b. Dedifferentiation: Nusbaum, 1912; Kenk, 1922; Steinmann, 1926; Prielgauskiene, 1933. See also Goetsch, 1929, 1931; Bandier, 1936, and the general papers; Schultz, 1908; Stolte, 1936. Also Curtis, 1940, "The histologic basis of regeneration and reassociation in lower invertebrates," *Amer. Nat.*, 74; Hyman, 1940, "Aspects of regeneration in annelids," *Amer. Nat.*, 74.

¹⁴ See, e.g., Hämmerling, 1924a, b; Probst, 1931, 1932.

cells."¹⁵ According to Probst (1932), the annelid *Owenia* regenerates without neoblasts. In short, neoblasts are apparently not necessary for annelid regeneration, and most of the evidence indicates that when they are present they are activated and dedifferentiated cells from the coelomic wall.

A similar difference of opinion exists concerning reconstitutive development of ascidians, either from winter buds or cell masses remaining after degeneration of zooids or directly from pieces of zooids or stolons. However, even though many cells degenerate and die when reduction of zooids or isolated pieces occurs, the chief reason for regarding the cells that remain and give rise to new individuals as undifferentiated seems to be the fact that they do remain and develop. In these cells form, appearance, activity, and relation to other cells undergo change in connection with the new development, apparently a regression to a less differentiated condition and a new progressive differentiation.¹⁶

Butler and, later, Thornton have presented very definite evidence of dedifferentiation in regeneration of the amphibian limb. Thornton maintains that the regenerating tissue, the blastema, originated by the dedifferentiation of cells of the limb stump.¹⁷ That dedifferentiation is involved in lens regeneration from the dorsal margin of the iris in adult amphibia appears beyond question. The iris cells lose their pigment, resume division and growth, and differentiate into a lens (p. 396). Retinal cells may also give rise to cells resembling lens cells. Changes suggesting dedifferentiation have been observed in various explanted tissues by many investigators, but some maintain that it never occurs. In cultivation of embryonic rat tissue on the chorioallantois of the chick there seems to be considerable dedifferentiation (Nicholas and Rudnick, 1933). Many other cases of apparent dedifferentiation might be cited.

General discussions of the subject show the same difference of opinion as special papers. Schultz (1908) and Nusbaum (1912) maintained that

¹⁵ R. G. Stone, 1932, "The effects of X-rays on regeneration in *Tubifex tubifex*," *Jour. Morphol.*, 53; 1933, "The effects of X-rays on anterior regeneration in *Tubifex tubifex*," *ibid.*, 54. See also L. H. Hyman, 1940, "Aspects of regeneration in annelids," *Amer. Nat.*, 74.

¹⁶ Schultz, 1907, 1908; Huxley, 1921a, 1926; Spek, 1927.

¹⁷ E. G. Butler, 1933, "The effects of X-radiation on the regeneration of the fore limb of *Amblystoma* larvae," *Jour. Exp. Zool.*, 65; 1935, "Studies on limb regeneration in X-rayed *Amblystoma* larvae," *Anat. Rec.*, 62. See also C. S. Thornton, 1938a, "The histogenesis of muscle in the regenerating fore limb of larval *Amblystoma punctatum*," *Jour. Morphol.*, 62; 1938b, "The histogenesis of the regenerating fore limb of larval *Amblystoma* after exarticulation of the humerus," *ibid.*

dedifferentiation occurs very often and have given many examples. Schaxel (1915, 1921) and Hämmerling are certain that it never occurs. Weiss (1939) makes a distinction between dedifferentiation, which he believes does not take place, and "modulation," which is believed to occur. However, it is not evident from his discussion that modulation is anything but a lesser degree of dedifferentiation. According to Schotté, dedifferentiation occurs in many cases.¹⁸

Doubtless; the differences of opinion will continue until we know more about what constitutes differentiation in any particular case. At present, however, there is a large body of evidence indicating that more or less dedifferentiation does occur in many organisms and many tissues, even to some extent among the higher vertebrates. Probably cells previously subjected to a developmental environment producing less stable differentiation are more capable of reaction to altered environment than others. Dedifferentiation does not necessarily involve complete loss of determination of cells but is alteration of structure and behavior in the direction toward the more general characteristics of cells of earlier stages of development. Such changes certainly do occur in many cells. The assumption that certain cells in adult organisms are undifferentiated, irrespective of their structure and function, because they are able to become, or give rise to, cells with other structure and function requires not merely assertion but rigid proof.

¹⁸ O. Schotté, 1939, "The origin and morphogenetic potencies of regenerates. First symposium on development and growth," *Growth*, Suppl. 1939.

CHAPTER IX

PHYSIOLOGICAL INTEGRATION: DOMINANCE AND PHYSIOLOGICAL ISOLATION

THAT the organismic individual is a more or less closely integrated whole is evident. Certain questions concerning origin and nature of the integrating factors, their limitations in some organisms, as indicated by physiological isolation, and their relation to developmental patterns are considered in this chapter. The examples are taken in large part from agamic and reconstitucional development because physiological isolation is often conspicuous in relation to these forms of development and more accessible to experiment than is usually the case in embryonic development.

It was pointed out in chapter i that physiological dominance or control may be effected either by transmission of energy changes or by mass transport by diffusion or otherwise of chemical substance. In earlier stages of reconstitucional and various other forms of development the dominant region is generally the "high" region of a gradient, and by inhibiting its activity the range and effectiveness of dominance is decreased and obliteration of the gradient obliterates dominance. It is often possible to establish a dominant region and a gradient in relation to it by establishing a localized region of increased activity. Moreover, in reconstitution in the simpler animals and in many other cases the central nervous system becomes an important factor in dominance. These and various other facts brought to attention in the following pages suggest that in the primitive form of developmental dominance the dominant region acts essentially like a region of excitation and that dominance of such a region is effected by transmission rather than transport. Such transmission is perhaps primarily electrical and a result of potential difference between the dominant region and other parts, or it may be transmission of a protoplasmic excitation in which electrical factors are undoubtedly concerned. In the simpler organisms this type of dominance is limited in range of effectiveness, but the range varies with activity of the dominant region and can be altered experimentally. The evidence indicates that there is a decrement in effectiveness with increasing distance from the dominant region and

that at a certain variable distance dominance becomes ineffective as a factor determining development or maintenance of parts already developed. In protoplasms lacking specialized conducting paths the effects of local excitation are apparently transmitted with a decrement; and even in cases in which some part of the central nervous system appears to be the dominant region, evidences of limited range of dominance often appear in the simpler animals; but whether this limitation results from an actual decrement in nervous transmission in primitive nerve complexes or from other factors—for example, incomplete differentiation of the nervous system in regions of rapid growth in length—is uncertain. Protoplasmic excitation and transmission attain their highest development in nervous tissue. The early development of the nervous system, the localization of the chief masses of nervous tissue in, or in close association with, the high regions of gradients, and the close parallelism between relations of dominance and subordination within the nervous system and gradients of earlier stages suggest that the nervous system is a relatively direct developmental expression of the primary factors in organismic integration.

A part of an individual which, for any reason, comes to lie beyond the range of effective dominance is physiologically isolated, that is, it is no longer subjected to the factors which were concerned in determining its development as a part of the individual or its persistence as a particular part. If the part is not so stably determined or differentiated that it cannot react to this isolation, it tends to lose more or less completely its characteristics as a definite part of the individual and may, under certain conditions, reconstitute a new individual.

This limitation in the effective range of dominance is a factor in limiting length of the individual or zooid in many organisms, but the limit of dominance and of length varies with conditions. An intensely active dominant region determines, in general, a greater length than one less active—for example, in various flatworms. Also, a greater length of individual or zooid is attained in planarians, *Stenostomum*, and various other forms with slow than with rapid growth, probably because differentiation of the longitudinal nerve cords in the regions of most rapid growth more nearly keeps pace with a slow, than with a rapid, increase in body length. In planarians and *Stenostomum* the length of a single zooid increases with advance in development of the head. If growth in length of a *Stenostomum* chain is rapid, a new fission zone and head region arise at a shorter distance from a zooid head in early developmental stage than from a fully developed head.

In the more highly differentiated animals range of effective dominance is not limited, except perhaps in earlier developmental stages. With attainment of all-or-none conduction of nervous impulses nervous dominance is effective over an indefinite distance. The limit of individual size in these forms is determined by factors limiting growth rather than by a limited range of dominance. Dominance effected by mass transport of substance is not necessarily limited in range, though it may be so limited if the substance decreases in concentration by spreading over a greater area or is progressively used up or altered in the course of transport.

In many of the simpler organisms physiological isolation with resulting reorganization and reconstitution of the isolated part may result from any one or more of four factors: (1) increase in length of the polar axis, so that a part comes to lie beyond the range of effective dominance; (2) decrease in effective range of dominance in consequence of decrease in activity of a dominant region; (3) blocking by local inhibition between dominant and subordinate region; (4) alteration of a subordinate part so that it becomes more or less insensitive to dominance, by activation or stimulation from some other source and probably in many cases by progressing determination and differentiation.

A sufficient degree of physiological isolation of a part may result in its development into a new individual, as in cases of budding in coelenterates and other forms and of fission in planarians and annelids. Reduplication of parts, segments, etc., from a growing region probably also involves at least partial successive physiological isolations. In general, agamic reproduction in axiate organisms appears to be a reconstitution resulting from physiological isolation, as reconstitution of pieces results from physical isolation.

Physiological isolation of a part by its determination or differentiation leads to a different result, that is, independent or self-differentiation. Moreover, attainment of a certain degree of specificity by a part may result in its activation in spite of dominance. The orderly appearance of localized regions of increased developmental activity, increased rate of dye reduction, and increased susceptibility in connection with development of particular organs, as in the chick embryo (pp. 159-63), suggests this sort of self-isolation. When the region from which the optic vesicle develops attains a certain physiological stage, it undergoes an activation irrespective of any general dominance. Similarly, the otic region, the appendage region, etc., undergo activation at a certain stage. Such physiological isolation may or may not be so complete that the part can con-

tinue its development unaltered when physically isolated, but it has certainly become so far isolated that it can develop in a way different from preceding stages.

Dominance of the primitive type may be effective in establishing a gradient and later in maintaining it, and so in determining the course of development of its different levels. Such a region is an inductor in earlier stages and later may determine persistence of the induced development. Even a part capable of self-differentiation after a certain developmental stage may be incapable of continued existence after differentiation without influence of a dominant region; muscular tissue, even in higher vertebrates, is an example.

In recent years much has been learned concerning the roles of specific chemical substances as factors of physiological dominance in development and of maintenance in adult life. As regards the great significance and the exceedingly complex interrelations of chemical dominance, there can be no question. However, most of the investigations in this field concern advanced stages of development or functional relations in mature individuals and, except for the recent work on plant hormones, are largely concerned with the higher vertebrates. It has often been pointed out that the earlier the stage of development the less evidence it affords of specific interrelations of parts or of chemical dominance. If quantitative gradients are the primary factors in axiate pattern, it follows that chemical dominance by production, transport, and effect of specific substance is not the primary form of physiological dominance but is possible only after different regions have become, to some degree, specifically different. The high region of a gradient may produce more of a certain substance than other levels, and its transport may alter the concentration of this substance at other levels and so influence their condition; but this is a nonspecific dominance directly related to gradient differences. A cell membrane may, in a sense, dominate the cell interior by its specific permeability to substances; but even in this case the membrane has become different from the interior in consequence of exposure to an external medium, and it is, in general, merely selective, not productive.

With origin of specific differences in development of the individual, chemical dominance becomes possible and with progress of differentiation evidently plays an increasingly important part in determining and influencing the further course of development, attaining its highest development in the hormone interrelations of the higher vertebrates. Even in these organisms, however, nervous dominance is still the chief integrating

factor and influences hormone production; but hormones, once produced, also influence the nervous system. It is perhaps of some interest to note that the hypophysis, apparently a highly important factor in the hormone complex, is, like the chief aggregation of nervous tissue, a development from the higher levels of the chief gradient.

In some cases transmissive and transportative factors may combine in dominance. A gradient established in the earliest developmental stages may influence direction of transport of chemical substance, as will appear in the following section. A transmitted nervous impulse may set free at the end of its path a particular substance, a "neurohumor," which determines the final effect. Physiological isolation from chemical dominance involves essentially the same factors as isolation from the primitive type of dominance: decrement in concentration or alteration with transport; decrease in production by the dominant region; blocking of transport; alteration of the subordinate part, making it insensitive.¹

DOMINANCE IN PLANTS

Extended discussion of plant dominance is beyond the present purpose, but attention is briefly called to a few points because of their interest in relation to dominance in animals. Experiments on plant dominance began with the early grower of plants, who learned to prune and trim in such manner that certain results were obtained; and the botanist, by extensive and varied experimentation, has thrown much further light on the problems concerned. The most familiar example of dominance in plants is that of the vegetative stem tip over lateral buds at stem-levels below it. In plants which give rise to lateral bud primordia (potentially new axes), the vegetative tip of the primary axis of some forms prevents the outgrowth and development of these buds, unless inhibited in activity or removed, but its dominance may decrease in the course of the life-cycle; in other forms it may retard their development and determine their growth form as lateral branches. The bean seedling is an example of the first type; the second type appears in many conifers and numerous other plants. In the bean seedling removal, inhibition of the tip, or blocking its effect results in outgrowth of the previously inhibited buds, those of the uppermost node reacting most rapidly and inhibiting more or less completely those of lower levels. In the conifer removal of the tip is followed by the turning-upward of one or more of the uppermost lateral branches and a change from the bilateral pattern of secondary branching character-

¹ See Child, 1921a; 1924b, chaps. x, xi; 1929b for earlier discussions of dominance.

istic of many conifers to the radial pattern of the primary axis. It is obvious that the growth forms of axiate plants must depend, to a great extent, on the degree or persistence of dominance of the primary stem tip and of other tips. If dominance is highly effective and persistent, the axis remains unbranched or may bear short lateral branches, usually different in pattern of secondary branching from the main axis. With less effective or decreasing dominance the plant may become a highly branched spreading form with several or many equivalent or nearly equivalent axes. Many trees and other plants show the first form in earlier stages; later, some degree of physiological isolation of branches evidently occurs, and some or all of them become more or less equivalent. Growing leaves, as well as stem tips, inhibit bud development. The root tip is apparently, also, to some extent a dominant region inhibiting formation of a new root tip within a certain distance or determining its development as a lateral root.

Various hypotheses concerning the mechanism of this dominance of stem tips and other active regions have been advanced. It has been held by some that there is in plants something analogous to nervous control; others have maintained that dominance is a matter of nutrition, the dominant region taking so large a part of the available supply that other parts are unable to obtain enough for their development; another view is that the dominant region produces substance inhibiting development of buds in other regions. Recently, however, discovery of the substances now known as "auxins" and the rapid development of analytic investigation concerning their production, distribution, and functions have thrown light on some aspects of the problem of dominance in plants.²

A biological method for comparative estimation of auxin amounts in terms of their effects on cell elongation has been developed with the coleoptile of the *Avena* (oat) seedling, which also played an important part in the discovery of auxin. The coleoptile is a sheath surrounding the young shoot. After an early stage its growth is by cell elongation without division, and the region of maximum elongation is some distance below the tip. Auxin is produced by the tip, is transported basipetally, and is concerned in the elongation. When coleoptile tips, stem tips, or other parts are placed on agar blocks, auxin, if present, diffuses into the agar; and if the block is placed on one side of a decapitated coleoptile, auxin is

² This field of investigation is developing so rapidly that any general survey is practically out of date by the time of publication. The book *Phytohormones* by Went and Thimann (1937) is the chief authority for the few points mentioned here. The book includes an extensive bibliography. See also Boysen-Jensen, 1936; Thimann and Bonner, 1938.

transported basipetally on that side, and cell elongation, with curvature of the coleoptile toward the opposite side, results. With standardization of the procedures involved and establishment of a biological unit, this method permits comparative assays of auxin activity with considerable accuracy.

Two auxins, A (auxentriolic acid) and B (auxenolonic acid), are distinguished; and indole-3-acetic acid (heteroauxin) has been found to act in the same manner as natural auxins. Many other organic substances, among them a substance from human urine, have more or less auxin-like action, and certain relations between chemical structure and activity seem to be established.

Auxins are apparently highly versatile substances. They, or inactive precursors which undergo activation, are produced in active stem tips, growing leaves, etc., and are transported, chiefly basipetally, in living tissues of plant axes. By differential distribution or production in relation to external factors and by inducing cell elongation they are concerned in tropisms. They induce root formation; in extremely low concentrations they accelerate growth of roots; but in higher concentrations they inhibit root growth. Apparently roots are either more susceptible to auxin than stems or do not require it for growth.

Auxin is transported rapidly in the living plant by a mechanism fundamentally different from diffusion but otherwise still obscure. Direction of transport in physiological concentrations is chiefly or wholly basipetal, that is, in a definite relation to the polarity of the axis concerned. This direction is maintained even against a concentration gradient of auxin. With concentrations far above the physiological range some acropetal transport may occur, probably in the transpiration current. With narcotization of coleoptiles by ether vapor, transport becomes essentially diffusion without polarity; with sufficiently low ether concentrations, this obliteration of polarity is reversible. At 0° C. transport also approaches diffusion but is still polar. It seems evident that the axiate pattern of vital activity is in some way concerned in auxin transport. Streaming of protoplasm, movement along interfaces, and electric potential gradients have been suggested as possible factors in directed transport, but none of these appears entirely adequate. According to Clark (1937), the electric-potential gradient can be obliterated experimentally without affecting polar transport, and this can be abolished with persistence of electric polarity. Association of auxin activity with oxidations is suggested by Thimann and Bonner (1938). That production and transport are associ-

ated in some way with cell metabolism and so with physiological gradient pattern seems to be indicated by the evidence.

As regards dominance of a stem tip, it has been found that in *Vicia faba* seedlings the stem tip produces the most auxin, the leaves less, and dormant axillary buds almost none; but these buds become producers when they develop. Auxin in agar applied to the cut end of the stem after removal of the tip is as effective in inhibiting axillary buds as the tip.³ Further experiment has given similar results with many other plants and with various auxins and substances showing auxin action. The mechanism of inhibition of axillary buds is still obscure. There is some evidence that auxin effect differs according as it moves with, or against, the polarity of the axis, being inhibitory when applied basally and moving acropetally and having the opposite effect when applied apically. If it passes from the stem into axillary bud axes, it moves acropetally in these axes. Certain lines of experiment indicate that inhibiting action of auxin may be indirect, through its effect on other factors.⁴

Physiological isolation of axillary buds can be brought about by inclosing the stem tip in an atmosphere without oxygen or in plaster, the tip remaining alive for some time under these conditions but auxin production being presumably inhibited. A zone of low temperature about the stem between tip and buds is effective in blocking dominance (Child and Bellamy, 1919, 1920). On the basis of these experiments it was suggested that a transmissive factor of some sort might be concerned. However, in the light of the data on the role of auxin in dominance and those showing that auxin transport is almost stopped by chilling a zone of stem below 5° C. (Cooper, 1936), blocking of dominance by low temperature appears to be a blocking of auxin transport; but these experiments also suggest that cell metabolism is in some way concerned in the transport.

This dominance of stem tips and growing leaves over buds which have already attained a certain developmental stage is a secondary, not a primary, type of dominance involving relations in a multiaxiate pattern already present. In the higher plants new axes, with leaf primordia as their first developmental expression, are localized in an orderly spatial pattern within the embryonic tissue of the tip itself. Each new bud appears at a certain distance from the apex of the tip and in a definite spatial relation to other buds already present. This spatial pattern shown

³ Thimann and Skoog, 1933, 1934; Skoog and Thimann, 1934.

⁴ See the discussion in Went and Thimann, 1937, chap. xii; also Went, 1936; Le Fanu, 1936; Snow, 1937.

by arrangement of leaf primordia (phyllotaxis) is species-characteristic under natural conditions—alternate, opposite, whorled, etc.—but often alterable experimentally.⁵ This pattern suggests, first, that a dominance of the extreme apical region of the main stem tip is effective over a very short distance in preventing new buds from originating; and that beyond that distance, at most a few millimeters, some degree of physiological isolation occurs, permitting initiation of lateral bud development and often continued development of a leaf; and second, that each actively developing bud or its leaf also dominates a certain area about itself and so prevents other buds from arising within this area, that is, each center of activity gives rise to a gradient system. That of the main tip has become axiate; that of the lateral bud is at first more or less radial, or symmetrical with respect to the axiate pattern of the stem tip, and becomes axiate by differential growth, like other buds. Nothing is known concerning the factors effective in determining and maintaining these orderly patterns within the stem tip. That they depend on auxin seems at present rather improbable. Doubtless, electric-potential gradients are associated with each growing primordium, but whether they are concerned in determining the spatial and chronological order remains to be determined.

The activity of the root system of higher plants inhibits more or less completely development of roots elsewhere, although physiological isolation may be brought about in many plants by subjecting other regions to conditions favorable to root formation. Experiment indicates that this general dominance of a root system depends largely on transport of water and salts from the roots. Depletion of these in other parts of the plant favors root formation, chiefly at the more basal levels of the stem, unless experimentally or otherwise inhibited there, presumably because depletion is most rapid there. This also is a secondary dominance depending on a pattern already differentiated. There is, however, some evidence of a local dominance with limited range at the more apical levels of a root axis. In the presence of an actively growing root tip a new root primordium appears only at a certain distance from the tip, and removal or inactivation of the tip destroys the dominance. Here, as in the stem tip, there appears to be some degree of physiological isolation with increasing distance along the root axis from the tip.

Evidences of dominance and physiological isolation also appear in the

⁵ See, e.g., Mary and R. SNOW, 1931, 1934; R. SNOW, 1929; and earlier work on phyllotaxis cited by these authors.

lower plants. For example, the very definite order of appearance, the rate, and the direction of growth of lateral branches of the thalli of many multiaxiate algae, even when the whole thallus is a single cell, as in *Bryopsis*, indicate presence of a definite spatial pattern involving dominance of the apical region of the main axis and physiological isolation at a certain distance from it. Development of new axes from cells that do not normally give rise to buds has often been induced by removal of the apical region in various algae and fungi. In the prothallia of liverworts and ferns dominance of the apical region has also been experimentally demonstrated. Many of these plants also afford evidence of decreasing effectiveness of dominance with increase in distance from the dominant region.

The so-called "gills" of the mushroom constitute a very definite spatial pattern; but when they are removed, leaving a flat surface, outgrowths may arise from any part of this surface, their localization being entirely irregular and apparently determined by local chance differences in activity. Each outgrowth dominates a certain area about itself, and an outgrowth somewhat in advance retards or completely inhibits development of other outgrowths within that area (Magnus, 1906); but beyond a short distance it is ineffective. Similarly, when numbers of adventitious buds develop near together, any one which develops more rapidly may retard or inhibit further development of others within a certain distance from itself. Whether dominance in these cases depends on auxin transport within the gradient system of each outgrowth or bud or on some other factor is not known.

DOMINANCE, BUDDING, AND AXIAL RELATIONS IN CERTAIN COELENTERATES

The hydra bud appears at the most proximal levels of the body capable of reacting rapidly to physiological or physical isolation. Removal of the apical region proximal to the tentacles accelerates development of buds. In animals in good condition buds appear only after the body has attained a certain length, and in a stock in uniform environment this length is fairly constant. Under depressing conditions, in "senescent" and in sexual animals, buds often appear at more distal levels than the usual budding zone, and more or less persistent "colonies" may result from delayed separation. After removal of the body distal to the budding zone a bud may inhibit reconstitution of a distal region and, with gradual change in position, replace the part removed. Apparently a developing bud inhibits

development of a second bud within a certain distance transversely in the parent body.⁶

The hydroid *Tubularia* gives rise to buds and branches to a varying degree in different species. The young animal, emerging from the gonophore, attaches to the substratum by its basal end; this spreads and becomes somewhat flattened; and, as the unbranched stem increases in

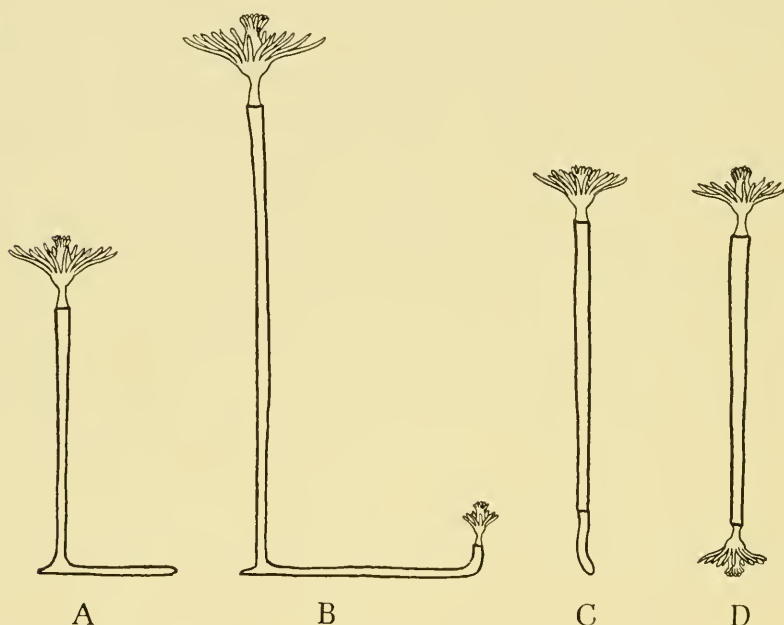


FIG. 108, A-D.—*Tubularia*. A, young individual with developing stolon; B, transformation of stolon tip into hydranth after physiological isolation by increase in distance from dominant hydranth; C, reconstitution of stem piece with hydranth distal and stolon proximal; D, bipolar reconstitution ("axial heteromorphosis").

length, one or more buds develop from the base and grow out as stolons. The stolon represents a new gradient, with high end at the tip, but differs from the stem in that it grows in contact with the substrate and remains a growth gradient without evidence of differentiation as long as it remains a stolon (Fig. 108, A).⁷ Both stem and stolon elongate; and when a certain length, varying with physiological and external conditions, is at-

⁶ The experimental literature on hydra is extensive. The following papers are in part directly concerned with the problem of dominance and budding: Hyman, 1928; Weimer, 1928, 1932, 1934; Rulon and Child, 1937a; they also give references to earlier literature.

⁷ Child, 1907a, 1919d, 1921d, 1923a; Child and Watanabe, 1935b; Watanabe, 1935c.

tained, the stolon tip alters its reaction, turns away from the substratum, and its tip becomes a new hydranth (Fig. 108, *B*). In relation to this hydranth a new stem develops, and later one or more new stolons grow out from its base, their tips becoming hydranths when sufficiently isolated physiologically from the dominant hydranth. In some species successive stems develop in this way at almost equal distances in series; in others the sequence is less regular. With still further increase in length of the original stem hydranth buds may arise in some species along the stolon and later from the proximal region of the stem itself. Each of these develops a stem and becomes a branch. Buds may also appear if the apical hydranth is removed and kept from reconstitution; but when it is present and in good condition, new hydranth development begins only at a certain distance from it. From the distal end of a stem piece several centimeters long a hydranth develops rapidly, and usually a second smaller hydranth develops more slowly from its proximal end (Fig. 108, *D*). Sometimes, however, particularly in certain species, a stolon appears at the proximal end (Fig. 108, *C*), and its tip transforms later into a hydranth. Stolons develop more frequently from the more proximal levels of the stem (Child, 1907*a*). In various hydroids apical regions can be transformed into stolons by inhibiting conditions (pp. 172-75): in *Tubularia* the stolon is evidently an axis somewhat inhibited by the dominant hydranth or by other conditions. It develops as a bud from the lower levels of the stem gradient in consequence of partial physiological isolation, and with increased isolation its tip becomes a hydranth. When the hydranth, the region of chief dominance, is removed, the degree of isolation at the proximal end of a piece may be sufficient to permit development of a hydranth there at once, or a short stolon may develop first (Fig. 108, *C, D*). So-called "axial heteromorphosis" in *Tubularia* differs from the natural agamic reproduction only in that removal of the dominant hydranth permits a sufficient degree of isolation for hydranth development at a shorter distance from the distal end than in the intact animal. In pieces the rate of hydranth development decreases from distal to proximal levels, except in very long pieces, in which the proximal end is already more or less physiologically isolated, and a rapidly developing oral hydranth retards still further the development of the aboral hydranth. This is shown by the more rapid development of the aboral hydranth when development of the oral hydranth is retarded or inhibited and also by the more rapid development of a hydranth at the distal end of a proximal piece than at the proximal end of the piece immediately distal to it. If oral and aboral hydranth develop-

ment begin about the same time, neither inhibits the other. The great mass of experimental data on reconstitution in *Tubularia* offers no difficulty to interpretation in terms of gradients and a dominance associated with them, with physiological and physical isolation possible, both experimentally and under natural conditions.

Corymorpha, also a tubularian hydroid, never gives rise to new hydranths by budding. The stolons are holdfasts, threadlike outgrowths, developing in large numbers from longitudinal series of buds in the basal region. Those nearest the basal end develop first, are dominant, and inhibit others until they have become so long that their dominance is not effective. On removal of the buds nearest the basal end by section, those adjoining the section grow out very rapidly and inhibit others. Each stolon is a gradient with high end at the tip (Child, 1928*b*; Child and Watanabe, 1935*b*).

In the reconstitution of stem pieces *Corymorpha* resembles *Tubularia*, except that the proximal fifth, more or less, of the stem, the only part secreting perisarc in mature animals, reconstitutes a basal region from its proximal end more frequently than *Tubularia*; this is according to expectation, since dominance in *Corymorpha* is evidently more effective than in *Tubularia*, as the absence of buds and branches shows. Pieces from the naked four-fifths of the stem reconstitute essentially like *Tubularia* pieces with hydranths at both ends, the proximal developing more slowly than the distal, except when pieces are so short that gradient difference is practically absent (Child, 1926*b*).

Watanabe (1935*c*) has made an experimental analysis of the dominance of the original hydranth and the development of dominance by a developing hydranth. With increasing delay in removal of the original hydranth its effectiveness in inhibiting hydranth development at the proximal end of the piece increases, as is shown by the increase in frequency of unipolar forms with the original hydranth at the distal end and proximal hydranth reconstitution completely inhibited. The experimental procedure and the result are shown in Figure 109. Frequency of unipolar forms increases from practically zero with section at both ends at the same time to 86 per cent with 72 hours delay of proximal section; that is, if the original hydranth remains 72 hours after the proximal section, it so completely inhibits hydranth development at the proximal end that, even after its removal, only 14 per cent of the pieces show hydranth development there, and most of the other pieces develop a basal end. If the original hydranth

does not remain in good condition, it is much less effective in inhibiting proximal hydranth development.

With removal of the original hydranth and delay of proximal section, unipolar frequency with hydranth at distal end increases from practically zero to 92 per cent with 24 hours delay and to 100 per cent with 48 hours delay of proximal section (Fig. 110); that is, if proximal section is made 48 hours later than distal, hydranth development at the proximal end is completely inhibited by the hydranth developing at the distal end. The

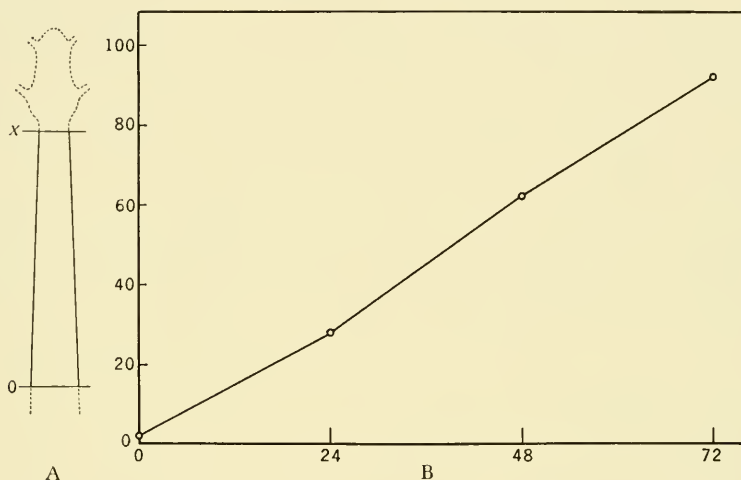


FIG. 109, A, B.—Dominance of original hydranth of *Corymorpha*. A, experimental procedure; proximal section at 0 hr., distal section at X (0, 24, 48, 72) hr.; B, graph of results; ordinates, percentages of unipolar frequency; abscissae, hours; fifty pieces in each lot (from Watanabe, 1935c).

developing distal hydranth is apparently more effective as a dominant region than the original, fully developed hydranth.

When distal and proximal section are made at the same time and the hydranth primordium is removed from the distal end after various periods of delay, unipolar frequency increases from zero with no delay to 70 per cent with 48 hours delay (Fig. 111). In this case, however, the hydranth is at the proximal end of the piece, hydranth development at the distal end is inhibited, and a base may develop there later; a complete reversal of polarity has resulted from localization of the dominant region at the proximal, instead of at the distal, end. In these pieces the dye-reduction gradient is also reversed. In general the dye-reduction gradient or gradients show close parallelism to the dominance, as indicated by hydranth

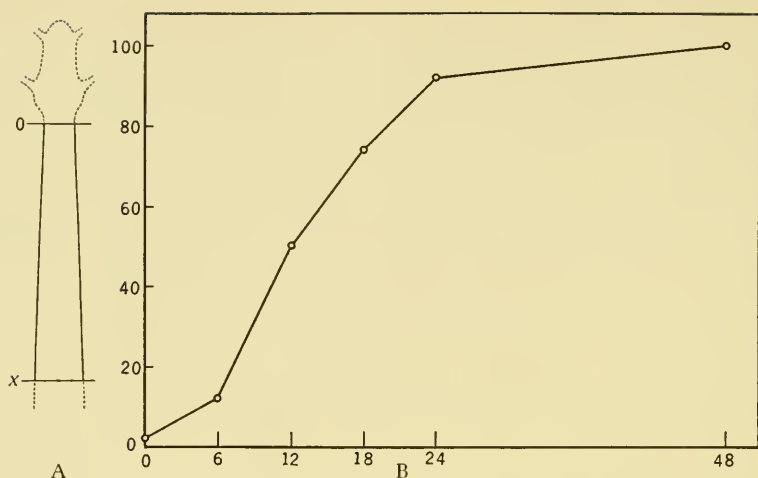


FIG. 110, *A*, *B*.—Dominance of developing hydranth of *Corymorpha* at distal end of piece. *A*, experimental procedure; distal section at 0 hr., proximal section at *X* (0, 6, 12, 18, 24, 48) hr.; *B*, graph of results; ordinates, percentages of unipolar frequency; abscissae, hours; fifty pieces in each lot (from Watanabe, 1935c).

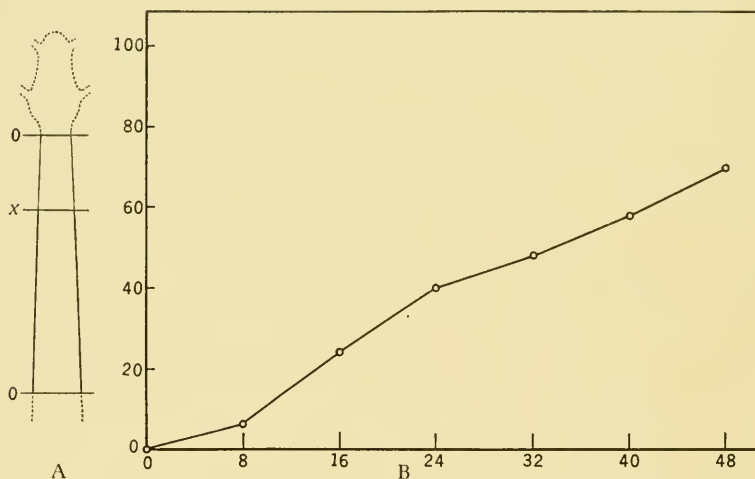


FIG. 111, *A*, *B*.—Development of dominance by hydranth of *Corymorpha* at proximal end of piece. *A*, experimental procedure; distal and proximal section at 0 hr., hydranth primordium at distal end removed at *X* (0, 8, 16, 24, 32, 40, 48) hr.; *B*, graph of results; ordinates, percentages of unipolar forms; abscissae, hours; fifty pieces in each lot (from Watanabe, 1935c).

development and inhibition. In the case of reversal of dominance and polarity (Fig. 111) the reduction gradient was found to be completely reversed in 86 per cent of pieces from which the distal hydranth primordium was removed 48 hours after isolation of the pieces, the dye-reduction test being made several hours later. In general the experiments along this line indicate that a little more time is required for attainment of complete dominance and reversal of the gradient by a hydranth developing at the proximal end than for attainment of complete dominance by a hydranth at the distal end.

A very similar increase in unipolar frequency appears in pieces of the stalk of the sessile scyphozoan *Haliclystus* with delay of proximal or distal section for different periods. With simultaneous distal and proximal section the stalk pieces show a high bipolar frequency (Fig. 113, *J-M*, p. 334), but this can be reduced to zero with a certain period of delay of proximal section and reversal of polarity in the whole piece can be brought about with increasing frequency in the same way as in *Corymorpha* pieces (Fig. 111). Moreover, gradients in the indophenol blue reaction a few hours after section show almost complete correspondence to the bipolarity or unipolarity developing later (Watanabe, 1937).

The branching hydroids show spatial and chronological orders very similar to those of multiaxiate plants. Many tubularian (gymnoblant) hydroids are monopodial, that is, the primary axis persists and the first hydranth retains more or less dominance, later buds giving rise to lateral branches in radial or spiral order about the main axis, or opposite or alternate in a single plane. At a sufficient distance from the dominant region or in its absence lateral branches may transform into main axes. In many species of campanularian (calyptoblant) hydroids the axial pattern is sympodial, that is, each new bud on each axis becomes temporarily dominant, is later subordinated to the next bud, and becomes a lateral branch of similar sympodial character. In some other hydroid species the axes are more or less equivalent, and the group resulting from budding consists of similar zooids (e.g., *Clava*). The spatial relations between dominant hydranths and new hydranth buds indicate a more or less definite range of effectiveness of dominance but varying with physiological and external conditions. In various hydromedusae other medusae develop from buds on the manubrium. Dominance and physiological isolation are evidently concerned in determining spatial and chronological order identical with that of phyllotaxis in certain plants. According to Wood-Jones (1912), the apical zooid is dominant and radial in form in certain branch-

ing corals; lateral zooids bud from about its base and are dorsiventral in the direction of the branch axis, or, perhaps more correctly, in the direction of the radius of the apical zooid in which the bud appears. At a certain distance from the dominant apical zooid a lateral zooid may transform into a radial apical zooid and become the apex of a new branch; or if the apical zooid is removed, one or more of the uppermost lateral zooids may become an apical zooid. The resemblance to the dominance of the stem tip over lateral buds in plants is evident, but the mechanism of dominance is undoubtedly different. In certain other coral species the zooids are equivalent; and in some an apical dominant zooid may be present in favorable, and all zooids equivalent in unfavorable, environment.

It is suggested that the nerve net is the chief factor in dominance in hydroids and other coelenterates; but whether the limited range of dominance in these forms depends on transmission with a decrement in the net or on the relation between rate of increase in length of the body and of differentiation of the net, the experimental data do not show. If the nerve net shares in the gradient difference along the stem of such a form as *Tubularia*, for example, there may be a transmission decrement from higher to lower levels. The time required for attainment of dominance by a new hydranth in reconstitution suggests that reorganization of the nerve net may be concerned.

A different conclusion has been reached by Barth (1938a). He finds that an oil drop introduced into the coenosarc of the *Tubularia* stem blocks dominance and concludes that the factor determining dominance is transported in the circulation in the gastrovascular cavity of the stem. If this is the case, the factor must be inactivated or disappear in the course of transport, for the range of dominance is limited, and under given conditions the limit is at a rather definite distance from the dominant hydranth. However, since *Tubularia* is highly susceptible to decrease in oxygen (Barth, 1938b) and the perisarc is not highly permeable to oxygen, it seems possible that the oil drop may decrease oxygen supply sufficiently in the coenosarc in contact with it to block transmission through the nerve net. Relations of dominance and physiological isolation are essentially similar in *Corymorpha* and *Tubularia*, but in *Corymorpha* a large number of small longitudinal canals lying just beneath the ectoderm represents the gastrovascular cavity of the stem. Regular or uniform circulation in these appears impossible because the naked stem contracts, extends, and bends almost continuously, even in isolated pieces; but localization, length, and time of appearance of hydranth pri-

mordia show a high degree of regularity. In the *Tubularia* stem and probably in *Corymorpha* circulation is in both directions. Why does not the circulating factor inhibit the dominant hydranth? Also, how is it possible for hydranths to develop simultaneously at both ends of a short piece without greatly inhibiting each other if each produces an inhibiting substance?

FISSION AND BUDDING IN FLATWORMS AND ANNELIDS

PLANARIANS

In certain species of the triclad genera *Planaria* and *Dugesia* (= *Euplanaria*) fission usually occurs in individuals above a certain length at a more or less definite body-level posterior to the mouth. Morphological evidence that a new individual is developing in the posterior region is usually lacking in planarians, but repetition of the copulatory organs in the posterior zooid region of *D. dorocephala* has been reported (Kenk, 1935a). Animals showing this duplication were prevented from undergoing fission by maintaining them at low temperature. According to Kennel (1883, 1888), cephalic ganglia, eyes, and a new pharynx develop before separation in the posterior zooid of a planarian from Trinidad. In *D. paramensis*, which divides anterior to the pharynx, a new pharynx develops in the anterior zooid preceding fission, suggesting some degree of physiological isolation of the posterior zooid.

In *D. dorocephala*, the material for most of the experimental work on fission, difference in susceptibility, respiration, dye reduction, head frequency, and motor reactions indicate that the region posterior to the fission zone represents one or more zooids, partially isolated physiologically from the dominance of anterior regions.⁸ There is no evidence of a posterior zooid in very small animals soon after hatching or in individuals reconstituted from short pieces, but with increase in length the posterior zooid region becomes distinguishable by various methods, though fission does not usually occur until animals are 15 mm. or more in length. With rapid growth in length the posterior zooid appears and fission takes place in shorter animals than when growth is slow, and with increasing degrees of inhibition of head development the length attained before fission decreases (Child, 1911d). Evidently the range of effective dominance increases with increase in length of the animal, but in general less rapidly than the length, so that progressive physiological isolation of the posterior region results.

⁸ See pp. 42, 43, 45, 109, 112, 181.



FIG. 112.—Elongation and permanent alteration of form in *Dugesia dorotocephala* resulting from repeated independent reaction of posterior zooid without fission.

The act of fission in large intact animals may begin with independent motor reaction of the posterior zooid region, consisting in attachment to the substratum, while the anterior zooid attempts to advance. In the struggle the body anterior to the fission zone often becomes greatly elongated until rupture results. Following rupture, both zooids undergo reconstitution. Fission sometimes results from sudden longitudinal contraction anterior and posterior to the fission zone. With slow growth in length in the laboratory animals may attain more than twice the usual length without fission; in these growth beyond a certain length is almost entirely in the posterior zooid region (see Fig. 16, p. 42). In many of them the posterior region shows independent reaction; but fission is not completed, either because the animals are not active enough or because the tissues rupture less readily than in smaller, younger animals. By frequent repetition of the independent reactions the posterior part of the anterior zooid becomes permanently much elongated and very slender (Fig. 112), and there is complete or almost complete absence of control of the posterior zooid region by more anterior levels. It is merely dragged about as the anterior zooid advances and often interferes with the advance by attaching itself, but it is usually detached sooner or later by the struggles of the anterior zooid or may release itself after the anterior zooid has ceased to struggle. In these animals physiological isolation of the posterior zooid has become directly visible.

Fission is readily induced by removal of the head, even in animals far below the length at which it usually occurs (Child, 1910a). Low concentrations of anesthetics and other inhibiting agents also induce fission, apparently by decreasing dominance; and a second division of the posterior fission piece often follows the first. Under these conditions fissions are usually limited to the period when the regenerating head has developed far enough to determine more or less motor activity but apparently has not attained full dominance. If there is no fission during this period, it usually occurs only after increase in length or another removal of the head. When animals with differ-

entially inhibited heads are fed, the length attained before fission is less than in animals with normal heads and decreases from teratophthalmic, through teratomorphic, to anophthalmic head forms (p. 178). Acephalic forms often divide near, or even anterior to, the middle when stimulated to contraction, though only 3 or 4 mm. in length, dominance being almost absent.

STENOSTOMUM

In the rhabdocoel families *Stenostomidae* and *Microstomidae* morphological differentiation of zooids precedes fission; and since increase in length is more rapid than differentiation and separation of zooids in well-fed, rapidly growing animals, new zooids develop before separation of earlier generations; consequently, long chains of zooids in various stages of development result (see Fig. 10, p. 27). Each zooid in the chain undergoes division when it attains a certain length, and in *Stenostomum* the length attained by any zooid before a new fission zone appears in its posterior region increases with advance in development of its head, but the length of a zooid when it first becomes distinguishable is approximately the same at different levels of the chain under uniform conditions. Apparently the length of body that a particular head can dominate increases as development of that head progresses. This relation is evident in Figure 10.

With each new zooid new cephalic ganglia develop and reorganization of the nerve cords posterior to each new head must result. It is probable that the progressive extension of dominance posteriorly with advance in head development is associated with progress posteriorly of this reorganization. In animals living at low temperatures with little food, consequently with slow increase in length, single zooids may become as long as well-fed chains at higher temperatures.

Stenostomum is a protandric hermaphrodite. When a chain attains the male stage, the zooids already present in the chain develop and separate, but there is no further development of new zooids. When the individuals attain the later female stage, they cease to feed and the pharynx degenerates; but they continue to elongate until they are as long as many of the agamic chains, but without development of new zooids. Apparently increase in range of dominance keeps pace with the elongation; it is less rapid than in well-fed chains. After section of the elongated females at postcephalic levels the posterior piece remains acephalic.⁹ In this respect

⁹ These observations and experiments are chiefly with *Stenostomum grande*, but other species show similar relations of zooids in the chains. Sexual individuals of other species have not been obtained by the writer. See Child, 1902; Van Cleave, 1929.

the female stage of *Stenostomum* resembles rhabdocoel species without agamic fission; heads do not regenerate from levels any considerable distance posterior to the cephalic ganglia in those species.

ANNELIDS

Agamic reproduction by transverse fission and, in some species, by budding appears rather commonly among annelids; zooid chains with morphological development of zooids preceding separation are formed in various polychete and oligochete species, and in the polychete syllids several types of budding appear. In *Aeolosoma*, *Nais*, and some other genera of microdrilous oligochetes, each zooid elongates, a new zooid arises in its posterior region, and the length attained by each zooid preceding this division is apparently related, as in *Stenostomum*, to the stage of development attained by the head region of the zooid. Development of each new head must involve more or less reorganization of the central nervous system posterior to it; consequently, the length of body dominated by a developing head region is probably correlated with progress of this reorganization, as suggested above for *Stenostomum*.

The position of the fission zone in the oligochete *Pristina longiseta* can be altered experimentally by nutrition, temperature, and condition of medium (Van Cleave, 1937). With conditions favoring rapid growth of new segments—abundant food, temperature of 20°–25° C., and fresh culture medium; also with good conditions at 10° C.—new fission zones appear at shorter distances from a head than with poor nutrition at 20°–25° C. or with previously used culture medium and good nutrition at the same temperature. In other words, under conditions permitting rapid growth physiological isolation occurs at shorter distances; under conditions unfavorable to growth, at greater distances from a dominant head region. Position of fission zones in various other species is probably similarly determined (see, e.g., Eckert, 1927). Among the polychetes, the syllids show various types of budding as well as fissions. Physiological isolation is apparently concerned in these, but experimental data are lacking.

AGAMIC REPRODUCTIONS IN OTHER ANIMAL GROUPS

Division in many protozoa is apparently nothing more than cell division, but in some of the axiate forms there are indications of dominance and physiological isolation. Cytoplasmic reorganization of the body of *Paramecium* into two zooids begins before nuclear changes are visible pre-

ceding division, and the location of the fission plane is apparently determined soon after the preceding fission and several hours before it becomes visible (Peebles, 1912). Under certain experimental conditions *Paramecium* forms chains of zooids (Hinrichs, 1927), and some protozoan species form chains naturally. Some of the ciliates that form multiaxiate complexes give rather definite evidence of dominance and physiological isolation in the localization and sequence of new zooids—for example, *Zoothamnium* (Fauré-Fremiet, 1930; Summers, 1938*a, b*). Some of the remarkable forms of agamic reproduction among the Suctoria present extremely interesting problems for the future; at present the determining conditions are quite unknown (see pp. 609–14). In other suctorian species forms of budding apparently essentially similar to those in other groups appear.

Budding sequences in bryozoa afford beautiful examples of definite spatial patterns, indicating dominance and physiological isolation, and reconstitution also indicates dominance in axial relations.¹⁰ Development of buds from stolons in sessile ascidians suggests that physiological isolation is a factor in their origin. The remarkable types of budding in the pelagic tunicates—for example, the migration of cell groups from the ventral to the dorsal stolon and the difference between median and lateral buds on the dorsal stolon of *Doliolum*, and the periodic arrangement of buds in blocks or “wheels” in certain of the Salpidae—also suggest existence of very definite spatial patterns of ordering and control of these phenomena; but here, too, experimental data are lacking.¹¹ The relation between agamic reproduction and regression of the original individual, as described by Berrill (1935) for various ascidian species, indicates that physiological isolation by decrease or elimination of dominance is concerned.

AUTOTOMY AND FRAGMENTATION

The triclad *Fonticola velata* kept at or above 20° C. with abundant food, on attaining a certain length, ceases to feed, the pharynx degenerates, and the body undergoes repeated fragmentation from the posterior end anteriorly. Fragmentation at each successive level is preceded by internal degenerative changes. The isolated fragments, usually less than a millimeter in diameter, encyst and, after extensive degeneration and re-

¹⁰ See, e.g., Brien, 1936, and his citations of earlier work; Brien et Huysmans, 1937, for patterns of budding; Otto, 1921, for axial relations in reconstitution.

¹¹ In this connection see M. E. Johnson, 1910; Ritter and Johnson, 1911.

constitution of organs, emerge as minute individuals. A considerable degree of control of fragmentation is possible. Animals kept at low temperatures increase in length slowly and do not fragment, even when they have attained much greater length than the fragmentation length at higher temperatures. Occurrence and course of degeneration and fragmentation are also influenced by character of food supplied.¹² It is not certain in this case whether physiological isolation initiates the degenerative changes at the posterior end or whether the degeneration results in physiological isolation, but the latter appears more probable. It is evident to observation that the posterior region about to separate as fragment has ceased to behave like a normal posterior end, shows little or no reaction to stimuli, and approaches spherical form.

Autotomy or fragmentation results from irritation in various nemerteans and some annelids (e.g., *Lumbriculus*), apparently in consequence of independent muscular contractions in different regions. Excitation of postcephalic regions evidently isolates them physiologically for the moment, and independent contraction and physical isolation follow. A local dominance of an excited region may perhaps be a factor in determining length of pieces, that is, excitation at one level may prevent independent reaction within a certain distance, which will probably differ with body-level and other conditions.

REDUPLICATION OF PARTS

An actively growing part—for example, a tentacle—is apparently able to inhibit development of another tentacle within a certain distance of itself; but, if two or more such parts develop simultaneously, they appear to have little or no inhibiting effect on each other. These relations appear very clearly in the scyphozoan *Haliclystus*. After removal of the margin of the umbrella by transverse section the eight tentacle groups and marginal organs appear simultaneously and develop rapidly, but after oblique section reconstitution at the more distal levels retards or completely inhibits that at more proximal levels (pp. 50–52 and Fig. 24). A similar dominance of distal over more proximal levels appears in the scyphistoma of *Aurelia*, though somewhat less effective. Tentacle development is greatly retarded at the more proximal levels of oblique section; but new tentacles appear at these levels and develop slowly, the normal number, sixteen, being gradually approached and perhaps attained finally (Child).

The activation at a certain stage of development of the segment-forming

¹² Child, 1913c, 1914d; W. A. Castle, 1928.

region in front of the anal segment in annelids suggests a partial physiological isolation from anterior dominance at this stage, and the repetitive formation of segments appears to be a reproduction resulting from some degree of physiological isolation of each segment primordium from the slight and short-range dominance of the growing region. The segment remains a segment, instead of becoming a complete individual, either because it is subordinated as it develops to anterior dominance, probably through the nervous system, or because capacity for head development is absent. In some annelids subordination to anterior dominance is evidently the factor maintaining segmental character, for, when more anterior parts are removed, a head regenerates from the segment or segments adjoining the level of section. In other species the segment remains essentially unaltered when more anterior parts are removed, but it is still able to react to removal of posterior parts by regeneration of a posterior end and a new segment-forming region. Successive physiological isolations in the mesoderm may be involved in segment formation in other segmented animals. Probably physiological isolation, perhaps local in ectoderm, mesoderm, or entoderm, plays a part in determining localization and order of various other reduplications of organ systems and parts. Such isolations may be temporary, occurring at certain stages of development and being followed by reintegration.

DOMINANCE AND PHYSIOLOGICAL ISOLATION IN THE FUNCTIONAL ACTIVITIES OF CERTAIN ORGANS

The ctenophore plate row affords an extremely interesting example of functional dominance and physiological isolation.¹³ Each row is a gradient with high end toward the apical (aboral) pole of the body, but each plate in a row is capable of independent movement. Ordinarily, the whole row is dominated by rhythmic impulses transmitted through the nervous system from the central nervous tissue about the apical pole. When the whole animal is subjected to anesthetics, cyanide, or various other depressing agents in gradually toxic concentrations, the impulses from the apical nervous system become less frequent and perhaps weaker, and in the plate row susceptibility to the toxic agent decreases from the apical to the oral end. After some time under the depressing conditions a new rhythmic impulse, independent of, and more rapid than, the depressed apical impulse, begins to appear in the less susceptible and less inhibited oral two-thirds, half, or less of the row, according to experimental condi-

¹³ Parker, 1905; Child, 1917*c*, 1921*a*, pp. 212-20, 1933*a*, and literature cited in these papers.

tions. This region is now physiologically isolated from the apical region, and the region of origin of the new impulse dominates all levels of the row oral to it. Later the continued toxic action of the agent may inhibit this dominant region; and a second physiological isolation, with again a new dominant region, may appear still farther orally in the row. In the long plate rows of *Mnemioopsis* three successive physiological isolations, each with its new dominant region, have been observed under inhibiting conditions.

These isolations are similar, as regards relation to a gradient pattern and a dominance, to establishment of posterior zooids in planarians and other forms, except that in the ctenophore isolation results from decreased dominance while in the planarian under natural conditions it usually results from increase in length but may be brought about experimentally by decrease in dominance.

On recovery from the toxic effect a gradual functional reintegration of the plate row takes place, the physiologically isolated regions being again finally subordinated to apical dominance. In *Pleurobrachia* the extreme oral end of the row may become temporarily dominant after certain degrees of toxic action and direction of transmission of impulse reverses over the whole row. This is a reversal of polarity by differential inhibition, but with recovery the original dominance and direction of transmission gradually reappear, replacing the reversed transmission step by step along the row.

Physiological isolation at any level of the row may also be brought about by blocking the impulse from the apical organ by low temperature or mechanical pressure or by direct stimulation which initiates impulses independent of the apical impulse. By continued mechanical stimulation at a particular level of the row a new dominant region can be established. Impulses initiated in this region are transmitted orally, that is, down the gradient, over the whole length of the row oral to the level of stimulation, but acropetally only a short distance at first; with continued stimulation acropetal transmission extends farther and may even reach the apical end of the row, obliterating the apical impulse. After cessation of the stimulation the dominant region established by it gradually disappears, and the whole row is again integrated under apical dominance.

The vertebrate heart resembles the ctenophore plate row in many respects as regards dominance and physiological isolation. In the fully developed functional heart the sinus region is the pacemaker, that is, the dominant region, and it develops from the high end of the primary heart

gradient. In early development, however, contraction begins in the ventricular region and appears progressively toward the sinus and finally at the sinus. This probably does not represent a progressive shift of dominance from the ventricular to the sinus region but results from the course of growth and differentiation of the myocardium (see, e.g., Copenhaver, 1939). Dominance of the sinus does not become evident until contractility has developed over the whole heart. If the sinus of the fully functional heart is inhibited, physiological isolation results, and a beat is initiated at a lower level. As in the ctenophore plate row, physiological isolation of subordinate regions with resulting independent contraction can also be brought about by blocking the impulse from the sinus by direct stimulation of the part concerned. By transplantation of the dominant sino-atrial region to the conus region in the embryonic chick heart *in vitro*, reversal of direction of beat and determination of ventricular rhythm by the transplanted sino-atrium have been brought about (Paff, 1936).

In animals with tubular heart and periodic, or occasional, reversal in direction of passage of contraction along the heart—for example, ascidians and various arthropods—the pacemaker or dominant region is not fixed in position (Gerould, 1931, 1933; Wolf, 1932). The end which is dominant at a certain time loses its dominance sooner or later, in consequence either of fatigue or of some other change in condition; and the other end, becoming physiologically isolated, becomes independent, initiates a rhythm, and becomes dominant and the pacemaker before the region originally dominant regains its original condition. Later the same decrease in dominance occurs in the new pacemaker, and reversal in direction of beat again results. As already noted, a similar reversal in direction of transmission in the plate row of the ctenophore *Pleurobrachia* can be induced by differential inhibition and recovery or by direct stimulation of subordinate regions. In differentially inhibited fish embryos the heart may remain tubular with reversible beat (Gowanlock, 1923). In all these cases with reversal of beat the heart gradient is apparently readily reversible, either periodically under natural conditions or experimentally by physiological isolation of a previously subordinate part in consequence of differential inhibition or of direct stimulation of the part.

At certain definite levels of the mammalian alimentary tract are regions which, under ordinary conditions, dominate levels posterior to them for a certain distance and may be more or less independent of each other or, under other conditions, may be subordinated to the dominance of more anterior regions or to the most anterior dominant region. Under pathological

or experimental conditions subordinate regions may become dominant, in consequence either of decrease in the pre-existing dominance or of irritation or direct stimulation of a subordinate region, and the direction of dominance may be reversed locally or over a considerable length of the tract. A more or less complete physiological closure of the morphologically completely open intestine may result from local reversal in direction of dominance and contraction produced by a local irritation or lesion. The regions normally dominant represent the high ends of gradients, as indicated most distinctly in the small intestine, by graded differentials in rate of rhythmic contraction in isolated pieces, rate of respiration, length of latent period, irritability, tone, etc.¹⁴ Here, again, the similarity to the ctenophore plate row and to the heart is evident, as Alvarez (1928, chap. vii) has pointed out. A similar functional dominance is apparently present in the ureter and will probably be found in various other elongated organs in which functional activity progresses in a definite direction.

CONCLUSION

The evidence from agamic reproduction in both plants and animals and from reconstitution of isolated pieces indicates that physiological dominance tends to maintain the individual, zooid, axis, or part as an integrated unit, while physiological isolation, like physical isolation, antagonizes this unity and paves the way for origin of new individuals, zooids, axes, or parts. For the new development of the physiologically or physically isolated part, however, a new dominance and establishment of a new gradient or gradients are necessary; and these changes are possible only when the isolated part, or some of its cells, are capable of reacting to the isolation by activation and attainment of a more generalized behavior and so of initiating the reorganization.

The importance of the nervous system in dominating and integrating activities in later development and mature life is a familiar fact. Its early differentiation and the localization of the chief aggregations of nervous tissue in the high regions of the gradient pattern present in early stages suggest that, as regards the general features of its pattern, the central nervous system represents the morphological expression of the higher gradient-levels and is physiologically a further development of the primitive type of dominance. The influence of the nervous system in maintaining

¹⁴ Most of our knowledge of these relations in the alimentary tract is due to Alvarez and his co-workers. See Alvarez, 1928, and papers cited there.

structure and determining regeneration need not necessarily be specific but may merely serve to maintain metabolism in the part concerned at a level which maintains or makes possible the development of a certain structure. For example, muscle may differentiate in the embryo, but later it atrophies in the absence of innervation because in the earlier stages the intrinsic metabolism of the cells is sufficiently intense to determine muscle structure but later is not sufficient to maintain it in the absence of innervation. In the simpler animals dominance is apparently chiefly or wholly nervous or neuroid, so far as axiate pattern is concerned. In the embryonic development of so-called "mosaic eggs" and of vertebrates the regional cytoplasmic differentiation of the egg at the beginning of development or chemical relations resulting from this differentiation may play a part in bringing about the dominance of the nervous system.

CHAPTER X

PHYSIOLOGICAL DOMINANCE AND ORGANIZATION IN RECONSTITUTION

PROGRESSIVE DETERMINATION OF POLARITY

DEVELOPMENT of bud and root axes in plants, of buds in animals, and the reconstitution of pieces shows that the new polar pattern is not established all at once but begins with the dominant region and extends progressively from it, as far as range of dominance, size of isolated piece in relation to scale of organization, or presence of other dominant regions and polarities permit. The conclusion seems to be justified that polarity itself develops progressively in these cases. At an early stage of reconstitution, budding, or fission the apical or anterior region may be the only part of the new axis present. Whatever the level of section in the stem of *Tubularia* or *Corymorpha*, the apical region of the hydranth originates adjoining that level, except under inhibiting conditions which prevent attainment of a gradient-level high enough for development of an apical region; then the level adjoining the level of section sometimes gives rise to a more proximal region of the hydranth, and the extreme apical region may be permanently absent. Always, however, the new axis develops from this level. Whatever the level of section in the planarian body, if head regeneration is possible, it begins at that level and determination of the new polar axis progresses posteriorly from it. In embryonic development the so-called law of anteroposterior development indicates that, even though regional cytoplasmic differentiations are present in an egg, a physiological dominance and gradient factors may determine the order of their developmental activity though polarity is already present.

That a new polarity may originate gradually and progressively from a dominant region in nonembryonic forms of development which involve determination of new pattern seems evident from the available data. Moreover, the evidence concerning origin and progressive extension of a new gradient from a dominant region is in accord with this view. And, finally, the evidence pointing to this conclusion has a very important

bearing on the question of the nature of polarity and developmental pattern in general. If polarity results from a molecular orientation or an "intimate structure" of some sort, it is not easy to account for the gradual, progressive origin of a polar axis and pattern, beginning at one end. If we assume some factor which determines molecular orientation or intimate structure progressively from one pole, that constitutes the primary polarity; and the molecular or other structural orientation is a resultant, an expression of polarity, like other features of polar pattern. But as regards determination and progressive extension of a dynamic gradient from an activated region, no assumptions are necessary, for these phenomena are visible or can be made visible by various methods. If a structural orientation or a localization of different substances is present along a polar axis, it seems highly probable that it results from, rather than determines, a physiological gradient involving dynamic factors. Without operation of such factors it is not evident how the changes in structural orientation or localization of substances required to account for changes in polarity under experimental and other conditions are determined.

CAN A DOMINANT REGION ORIGINATE INDEPENDENTLY
OF OTHER PARTS?

If gradual progressive determination of a polarity, beginning at a dominant region, does take place, the dominant region must originate independently of other parts of the polar pattern. That it does originate independently in various kinds of buds and in many reconstitutions was briefly pointed out above. But the question whether it can develop independently of other parts of the polar axis, whether it is a self-differentiating system, remains to be considered. Among animals the most striking evidence of a high degree of independence is found in the partial unipolar, bipolar, and multipolar forms in hydroid, planarian, and annelid reconstitution. When short pieces of *Tubularia* or *Corymorpha* develop into partial forms, these, except occasionally under inhibiting conditions, represent the apical region and more or less of the normal polar pattern, according to length of piece, scale of organization, and presence or absence of other partial polarities (Fig. 113, *A-I*).¹ The parts of the axis formed in such cases appear normal in pattern, fully developed, even though they consist of nothing but a hypostome or a hypostome with distal tentacles. In partial forms developing from aggregates of dissoci-

¹ For other bipolar and multipolar forms see Fig. 15, *B*, p. 42; Fig. 116, p. 346; Fig. 120, p. 360; and Fig. 124, p. 366.

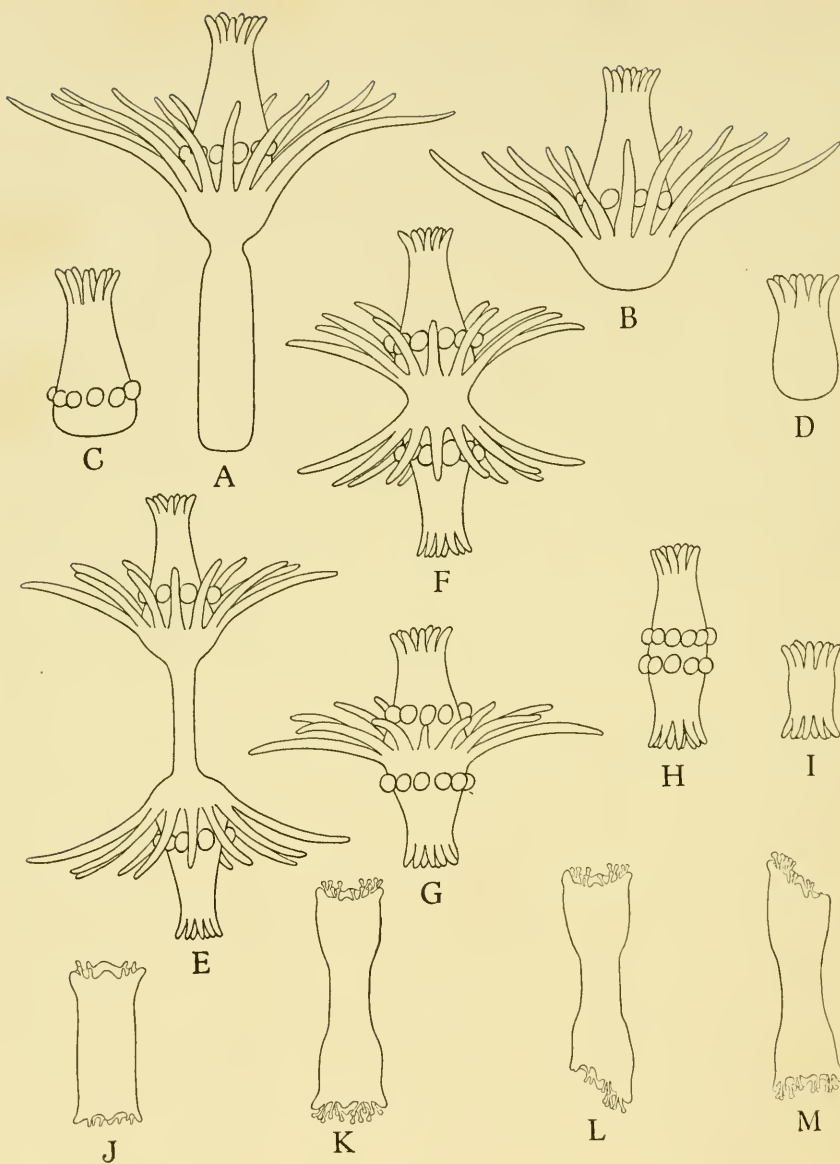


FIG. 113, *A-M*.—Unipolar and bipolar coelenterate reconstitution. *A-I*, apical partial forms of *Tubularia* or *Corymorpha* from pieces of different length (from Child, 1915c, and unpublished data); *J-M*, bipolar forms from stalk of the sessile scyphozoan *Haliclystus auricula* (from Watanabe, 1937).

ated *Corymorpha* cells the apical region shows a similar independence of other parts (Fig. 117, p. 347). It is possible, of course, that these partial apical forms fall short of complete development in some, as yet unrecognized, structural or functional characteristic, but no such lack is evident. The hypostome reconstituted from a short piece is capable of reacting to, and taking in, food, although the food immediately passes out at the proximal end.

Figure 113, *J-M*, shows interesting bipolar forms reconstituting from the stalk of the sessile scyphozoan *Haliclystus* (see Fig. 23, p. 50). The stalk differs rather widely in structure from the umbrella, but tentacle groups and marginal organs develop directly from the healed surface of section, and with simultaneous distal and proximal section frequency of bipolar forms is high (Watanabe, 1937). Further changes involved in reorganization of the stalk into an umbrella are much less rapid; and sometimes dominance is too weak to bring them about, and the piece remains essentially a stalk with tentacle groups at one or both ends (Child).

It has already been pointed out that a hydranth or head, reconstituting at the distal or anterior end of a piece from any level except that immediately adjoining the original hydranth or head, is "out of place," as truly "heteromorphic" as a hydranth or head developing at the proximal or posterior end of a piece. At either end they are hydranths or heads of new individuals and determine reorganization of pattern over a greater or less distance. When the hydranth or head develops at the distal or anterior end of the piece, the new polarity is in the same direction as the old and determines development of parts apical or anterior to the level of origin of the piece; but when it develops from the proximal or posterior end, it determines a polarity opposite in direction to that originally present. The evidence supports the conclusion that hydranth or head is not determined by other parts of the piece, for both can develop when other parts are not present or in a relation to other parts quite different from the normal. On a postoral planarian piece, for example, a head begins to develop before pharyngeal, oral, and prepharyngeal levels. If reconstitution were determined anteriorly from more posterior levels, we should expect that oral, pharyngeal, and prepharyngeal regions would develop successively, and finally the head; but this is never the case. The same relations appear in annelid reconstitution. Whatever the level of section, in species capable of head regeneration a head regenerates first, and in most species only a certain number of segments characteristic for the species regener-

ates posterior to the head, even though more were removed (e.g., Hyman, 1916a). In general it appears that a necessary condition for hydranth or head formation on an isolated piece is an activation of the cells concerned sufficiently intense to bring about a high degree of physiological isolation from other parts. When they become independent of other parts, they reconstitute a new dominant region and so begin development of a new individual. Any level of the *Tubularia* or *Corymorpha* stem, any postcephalic level of the planarian, and all, or the more anterior, postcephalic levels of many nemerteans and annelids will develop as hydranth or head if not dominated by some other part. In other words, so far as other parts of the body are concerned, the hydranth, or even its apical portion, and the head, or probably the nervous tissue of the head, appear to be self-determining and self-differentiating systems. They may be regarded as expressions of the primary or fundamental action system of the species, at least so far as reconstitution is concerned. In the reconstitution of various minor or subordinate and specialized axes, such as appendages of various sorts, relations as regards the longitudinal or polar axis of the part are apparently essentially similar, at least in earlier stages. The distal tip of the starfish arm regenerates "out of place" as regards relations to other parts and basipetal determination of the axis follows.² Regeneration of a whole distal part of an amphibian limb may take place from a half-transverse section (p. 370), and a distal part may even regenerate from a proximal surface of section of a limb implanted with longitudinal axis reversed (p. 390).

Position of a hydranth or head at the distal or anterior end of a piece may be determined by the original polarity of the piece, though it is not necessarily so determined; but the polarity of the developing hydranth or head is evidently independent of the original polarity, even when in the same direction, for it does not "fit" into that polarity but makes it over more or less completely, according to body-level of the piece. The original symmetry or asymmetry of the piece may play a part in determining symmetry or asymmetry of the reconstituting dominant region (pp. 387-89) but does not necessarily do so, for radial symmetry of hydranth and dorsiventrality of head may develop in various positions with respect to the original symmetry.

The actinian *Harenactis* affords interesting examples of development of symmetry in reconstitution. Partial disks develop after partial transverse section of body wall and esophagus (pp. 387-88); here radial pattern of

² King, 1898, 1900; Dawydoff, 1901; Morgulis, 1912; Schapiro, 1914.

the reconstituted partial disks and the complete disk at the distal end after complete transverse section are evidently directly determined by the original radial pattern. Under other conditions, however, reconstitution of a radial or of a bilateral pattern is possible in this animal quite independently of the original radial pattern (pp. 371-74). In planarians the anterior tip of a head regenerating from a complete transverse section is directed away from the piece, and its dorsiventrality coincides with that of the piece; but a complete head may regenerate from a surface of section half, or less than half, the width of the body or even from a longitudinal cut surface (Beyer and Child, 1930). A complete head may also regenerate from a partial transverse section in various annelids (Morgan, 1902*b*; von Haffner, 1931, etc.).

Von Haffner's experiments on *Lumbriculus* are of special interest. Following removal of lateral pieces from different body-levels with injury of axial organs, heads developed from the more anterior levels, both heads and posterior ends from middle levels, the posterior ends anterior to the heads, and only posterior ends from posterior levels (Fig. 114). In the middle region, where both heads and posterior ends develop, the heads decrease in size and development and posterior ends increase in size from more anterior levels of section posteriorly (Fig. 114, X, Y, Z). In this region posterior ends are anterior to heads because heads develop from the cut surfaces facing anteriorly, posterior ends from those facing posteriorly. As von Haffner suggests, these relations are doubtless due to the polarity of the original animal, but it is perhaps possible to go a step farther and suggest that differences in degree and probably in rate of activation and in dominance at different body-levels are concerned. At the more anterior levels the regenerating head dominates the new tissue completely, and only an anterior end develops. At the middle levels dominance of the regenerating head is less effective, and the dominance of the original ani-

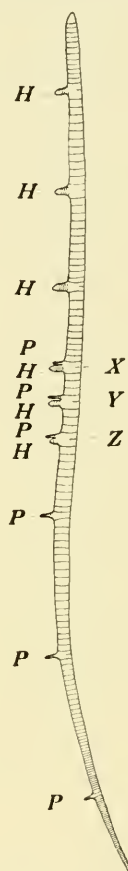


FIG. 114.—*Lumbriculus variegatus*, diagrammatic, indicating character of regeneration in relation to body-level after removal of small lateral pieces with injury of axial organs; H, head; P, posterior end (after von Haffner, 1931).

mal determines tail development; and, as activation and dominance of the tissue regenerating head decrease from *X* to *Z*, tail development increases because not antagonized so strongly by dominance of the developing head. At more posterior levels activation and dominance of regenerating tissue are not sufficient to overcome the original dominance, and only posterior ends develop. Both heads and posterior ends, however, though developing from partial transverse sections, are dorsiventrally and bilaterally complete.

In general, the polarity of a regenerated part, head, posterior end, or appendage may coincide with, or be oblique or opposed to, that of the original body; but the symmetry or asymmetry is usually in the same direction as the original and may be complete, though regeneration is from a partial section. If physiological axes are gradients, these features of reconstitution are very simply accounted for. There is no polarity differential on a transverse section; consequently, polarity in the reconstituting part is determined by the gradient arising in relation to activation following section and outgrowth. On a section oblique to the original polar axis there is a polar differential, and reconstitution in relation to oblique section is often asymmetrical (pp. 50-53). Ventrodorsality and dorsiventrality do present a differential on a transverse plane of section, and this must influence reconstitution from that plane; also, a partial differential on the plane of section may be sufficient to initiate development of the whole differential in the reconstituted part.

THE ORIGINAL NERVOUS SYSTEM IN RELATION TO NEW DOMINANCE

Much experimental work has been done in the attempt to determine whether or to what extent the nervous system of the original animal is a determining or essential factor in reconstitution. The fundamental question, as regards this problem, concerns the role of the nervous system in determining a new dominant region, or, more specifically, the new nervous system in that region. For example, do the parts of the nerve cords present in a headless planarian piece play a part in determining the cephalic ganglia in the regenerating head?

In hydroid reconstitution the hydranth is determined basipetally from the apical region; and since reorganization in a short piece may involve the whole piece in formation of the apical portion of a hydranth, irrespective of level of origin of the piece, it is highly improbable that the part of the nerve net originally present in the piece plays any essential role in

the reconstitution. Moreover, an aggregate of dissociated cells of *Corymorpha* and other hydroids can reconstitute a complete individual. Rate of regeneration of the scyphomedusa *Cassiopea* is decreased by removal of the marginal sense organs; but this is believed to indicate merely a nervous influence on the general metabolic level, since respiration is also decreased by removal of the sense organs (Cary, 1916).

As regards head regeneration in planarians, Flexner (1898) and Keiller (1910) have maintained that the cephalic ganglia may develop independently of the nerve cords in the piece. In a land planarian the new ganglia are said to develop in continuity with the anterior ends of the old nerve cords (Bandier, 1936). However, the distance between the cut ends of the nerve cords and the locus of the new ganglia is so small and nerve fibers so difficult to distinguish in planarians that a definite conclusion seems impossible. Even if the ganglia develop in continuity with the nerve cords, it does not necessarily follow that their development is determined by the cords; the ganglionic development apparently determines reorganization of the region of the nerve cords where it develops. The fact that heads can develop from a partial transverse or even a longitudinal cut surface lateral to the nerve cords leaves little doubt that ganglia can develop independently of the old nerve cords, though lateral branches of the cords, if present at the cut surface, may play a part in localizing head development. The ganglia of the regenerating head of the nemertean *Lineus* develop in complete independence of the old nerve cords and later become connected with them by outgrowth of fibers from the ganglia; in some other nemerteans the ganglia develop at the anterior end of the cords (Coe, 1934*a, b*).

It seems to be conclusively established that in the earthworm and some other annelids head regeneration can occur in the complete absence of the nerve cords at the cut surface.³ Nevertheless, the old nervous system may play a part in localizing head regeneration. When the ventral cord is present at the cut surface, it is evidently a factor in localizing head regeneration in annelids.⁴ Regeneration of a posterior end does not take place or is incomplete in absence of the nerve cord, and implanted pieces of cord may localize posterior regeneration.⁵ In view of the data, it is

³ Goldfarb, 1909, 1914*a*; Siegmund, 1928; Bailey, 1930, 1939; Avel, 1932; Kropp, 1933; Okada, 1934; Crowell, 1937; Painter, 1938.

⁴ Morgan, 1902*b*; von Haffner, 1931; Zhinkin, 1935; Sayles, 1937. See also Hyman, 1940, "Aspects of regeneration in annelids," *Amer. Nat.*, 74; Sayles, 1940, "Buds induced by implants of posterior nerve cord, etc.," *Biol. Bull.*, 78.

⁵ G. E. Holmes, 1931; Zhinkin, 1936; Sayles, 1939.

evident that the old nervous system is not an essential factor in establishment of a new dominant region in many species of hydroids, planarians, nemerteans, and annelids.

In certain triclads (e.g., Dendrocoelidae) and in various nemerteans and annelids head regeneration occurs only anterior to a certain body-level, characteristic for the species. So far as data are available, those rhabdocoels that do not undergo fission, polyclads, and some nemerteans and annelids do not reconstitute cephalic ganglia unless some portion of the original ganglia remains; that is, determination of an entirely new dominant region apparently does not occur.⁶ We do not know whether, or to what extent, nonnervous cells are concerned in regeneration of cephalic ganglia in these cases, but it is evident that only cells of the more anterior body-levels, whether nerve cells or others, are able to give rise to ganglia under known experimental conditions. This inability to regenerate ganglia and head does not mean that no cells capable of regeneration are present, for complete posterior regeneration occurs at levels incapable of head formation. It may be suggested that rate or intensity of activation following section at these levels is not sufficient to render the cells independent of other parts, and so to make possible development of new ganglia and head, but is sufficient to permit development of posterior ends under the dominance of the old nervous system.

The hypothesis that absence of head regeneration posterior to certain body-levels is due to decrease in number of undifferentiated "formative cells" from anterior to posterior levels fails to account for the rapid and extensive posterior regeneration at those same levels.⁷

Presence of the radial nerve at the cut surface, even if it is separated by section farther proximally from the rest of the nervous system, is essential for regeneration of the ophiurid arm, according to Morgulis (1912). Schapiro (1914) finds that regeneration of the distal part of the starfish arm is possible even when a rectangular piece, including both dorsal and ventral body wall, is removed at a more proximal level of the arm, leaving only lateral connection with other parts. In this experiment a piece of

⁶ For examples see the following: F. R. Lillie, 1901, a dendrocoelid triclad; Child, 1904b, 1910b; L. V. Morgan, 1905, the polyclad *Leptoplana*; Coe, 1932, a nemertean; T. H. Morgan, 1897, 1902b; Hyman, 1916a; Sayles, 1936, annelids. Various other papers give similar data, and unpublished data on a number of rhabdocoel species (Child) show absence of head regeneration in absence of ganglia.

⁷ For this hypothesis see Curtis and Schulze, 1924; Curtis and Hickman, 1926; Curtis, 1928. Steinmann, 1926, describes extensive dedifferentiation in planarian reconstitution, and Bandier, 1936, is unable to distinguish "formative cells" from other parenchyma cells in the reconstitution of land planarians.

the radial nerve remains at the distal cut surface but is isolated from central parts, except for possible lateral connections. Whether the nervous system is essential to regeneration of a complete disk and arms from a single arm in certain starfishes (e.g., Kellogg, 1904) is not known.

Reconstitution of the bryozoan from a statoblast and of certain ascidians from winter buds seems to be essentially development of a new individual from what is primarily little, if anything, more than a cell aggregate; the new nervous system develops in complete isolation from the old.

The question of nervous influence on regeneration of amphibian appendages has been the object of extensive experiment, and the literature shows marked difference on the conclusions reached, although most authors find some nervous influence.⁸ Some find merely retardation of regeneration and failure to attain normal size after elimination of regions of the spinal cord innervating the leg, and it has been repeatedly suggested that this effect is due merely to lack of function in the regenerating leg. However, the possibility of some degree of innervation by other than the normal nerves has not always been excluded. Goldfarb denies nervous influence. Walter finds that absence of motor innervation is without effect but holds, as does Locatelli, that regeneration does not take place in absence of nervous connection and suggests that connection with spinal ganglia is essential. According to Weiss, there is proliferation but no true regeneration in absence of nervous connection. He regards the nervous influence not as formative or determinative but as a tonic excitation from the sympathetic system. Schotté's experiments lead him to a similar conclusion. Hamburger, working with anura, finds legs normal in form but of small size, regenerated in apparent complete absence of innervation, but admits that the possibility of sympathetic innervation is not absolutely excluded.

At present there is general agreement that absence of motor innervation does not prevent leg regeneration but may retard or prevent growth to full size through absence of function. The experiments of Weiss and Schotté indicate the necessity of sympathetic innervation, and these and various other authors agree that the nervous influence is not formative. Apparently it is a factor in determining and maintaining the physiological state, the level of physiological activity, of the cells which makes possible a sufficient activation following section to bring about the degree of physiological isolation necessary for establishment of a new dominance

⁸ Goldstein, 1904; Wintrebert, 1904; Goldfarb, 1909; Wolff, 1910; Walter, 1911; Weiss, 1925*a*, 1930, p. 124; Schotté, 1926*b*, *c*; Brunst, 1927; Hamburger, 1928; Locatelli, 1929, and literature cited by these authors.

and gradient in the outgrowing tissue. That the nervous influence is not specifically formative is shown by experimental alterations of the course of brachial or sciatic nerves. Nerves thus diverted may determine limb development at other than the normal position within the limb field; but the sciatic nerve, led into the tail region, determines a tail.⁹ Transplanted primordia of chick appendages develop irrespective of innervation (Hamburger, 1939), but this development does not involve origin of a new dominance.

SERIAL HETEROMORPHOSIS OR HOMEOSIS

Among arthropods regeneration of an appendage characteristic of a particular segment usually results in an organ more or less similar to that removed, though perhaps not completely normal; but sometimes an appendage resembling more or less closely that of another segment, usually the next posterior, develops.¹⁰ In the case of development of antenna-like structures in place of eyes, as observed in various Crustacea, Herbst, Janda, and Kříženecký find that this type of regeneration occurs after removal of the optic ganglia, even though only the distal part of the eye stalk is removed. Removal of antennae near the base apparently favors regeneration of leglike structures; but, according to Suster (1933), normal regeneration of antennae is possible in the orthopteran *Sphodromantis* after removal of the ganglia. In all these cases the regenerated structure, when not normal, resembles the appendage of a segment posterior to that on which it regenerates. In the light of the data on reconstitution in hydroids and planarians and on differential modification of development by external agents, it may be suggested that here, also, degree or intensity of activation of cells following section is an essential factor in determining the segmental character of the regeneration. If the ganglion most directly related to the part is present, it is probably a factor in bringing about sufficient activation to determine normal segmental regeneration; in absence of the ganglion there is less activation and in many cases no regeneration, or an appendage may regenerate, but its cells fail to attain the physiological level necessary for development of an appendage normal to the segment concerned, and a structure resembling

⁹ Locatelli, 1925; Guyénot et Schotté, 1926. Guyénot, 1928, reports similar results in a reptile, *Lacerta*.

¹⁰ Antenna-like organs in place of eyes: Herbst, 1896, *b*, *c*, 1899, 1901*b*; Janda, 1913; Kříženecký, 1913. Leglike organs in place of antennae: Friza and Przibram, 1910; Przibram, 1910, 1917, 1919*a*, *b*; Brecher, 1924; Borchardt, 1927; Suster, 1933. Crayfish legs, Zalpeter, 1927. Homeosis in general, Przibram, 1910, 1920.

the appendage of a segment representing a lower level of the polar gradient of earlier stages results. Further evidence that degree of activation following section is concerned is the increasing frequency of homeosis with advancing age of the animal and its occurrence at low, but not at high, temperatures in certain cases. Metabolic rate and regenerative potency decrease, in general, with advancing age in postembryonic or postlarval stages and certainly decrease with decrease in temperature.

According to Zalpeter (1927), however, regenerating legs of the crayfish may show characteristics of either more anterior or more posterior legs.¹¹ The similarity of the crayfish legs suggests that gradient differences in the leg segments are not great and that slight variations in activation following section may be sufficient to determine development of a more anterior or more posterior leg on a certain segment. Moreover, a secondary gradient with high end posterior is probably present at certain developmental stages of the crayfish; if so, more anterior legs probably represent lower levels of this gradient, and their development from more posterior segments may also result from unfavorable or inhibiting conditions.

After removal of uropods and caudal ganglion Herbst (1917) found no regeneration of uropods in most cases; but in a few individuals uropods were recognizable, even though the caudal ganglion was not reconstituted. This is not a case of homeosis; but, like those cases, it suggests individual differences in degree of activation at the cut surface. Occasionally it is sufficient to determine uropod development in absence of the ganglion. Differences in physiological age or other differences in physiological condition, variation in degree of injury, manner of healing, etc., may account for the varying results. Supposedly, the cells concerned in homeotic regeneration are determined as appendage cells, perhaps as appendage of a more or less limited number of segments; but they are evidently not fixedly determined as cells of the appendage of a particular segment.

DOMINANCE AND SCALE OF ORGANIZATION

By "scale of organization" is meant the spatial order of magnitude of the developmental pattern in relation to an axis. In reconstitution of hydroids, planarians, nemerteans, annelids, and various other forms scale of organization may vary greatly with physiological condition of the original individual, with body-level from which the part is isolated, and with

¹¹ Przibram mentions cases of replacement of posterior by an anterior wing in insects and some other cases of appendages characteristic of more anterior segments, but conditions of origin of these are unknown.

natural and experimental environmental conditions. Proportional scale of different pattern components may also show similar variation (see chaps. v-vii). Reconstitution of parts of eggs, of isolated blastomeres or blastomere groups, and of parts of later embryonic stages shows in some forms considerable variation in scale of organization with size of isolated part; but experiments directed toward alteration and control of scale are few. In many eggs scale is so stably determined at the beginning of embryonic development that no great alteration appears under conditions thus far employed. Although data of many authors show differences in scale, their importance for problems of embryonic, as well as reconstitutive, development seems not to have been fully recognized.

At present it appears difficult to account for many of the expressions of scale of organization except in terms of dominance and gradient pattern. According to these terms, a gradient established in an isolated part in good condition will, in general, represent a more intense activity at its high end, will extend over a greater distance, and will perhaps be less steep than one established under unfavorable conditions; consequently, the local fields developing along its course, within which the particular parts develop, will also be longer and the scale of organization therefore larger. Such an interpretation must, of course, remain general until we know more about metabolism and its changes at different levels of a physiological axis in reconstitution and embryonic development and about the relation of dominance and determination to such factors. A localized active region, however it originates, may determine a gradient or gradient system and so become a dominant region, or a gradient may arise as a direct reaction to an environmental gradient. In the latter case the environmental gradient is merely the initiating factor; protoplasmic constitution and condition are the factors determining the final character of the gradient; and the high end apparently is more or less dominant. Dominance and the gradient establish a physiological basis for definite axiate pattern. In short, the dominant region is or may be an inductor or "organizer," that is, it determines the organization or reorganization of other parts. A few examples of alteration of scale of organization in reconstitution will serve to indicate some of the relations between scale and certain factors.

SCALE OF ORGANIZATION IN HYDROID RECONSTITUTION

Many investigators have noted the great variation in length of the hydranth primordium in reconstitution of stem pieces of *Tubularia*.¹² The

¹² See, e.g., Bickford, 1894; Driesch, 1897, 1899; Morgan, 1901b, 1902a, 1903a; Child, 1907a.

formation of this primordium and its decrease in length from distal to proximal stem-levels were discussed in chapter ii. Physiological condition of the individual from which the piece was taken is also a factor in determining primordium length. Pieces from animals in good condition, as indicated by size and activity of the original hydranth, generally develop longer primordia at given stem-levels than pieces from animals with small degenerating hydranths or none. Primordium length can also be altered by inhibiting and accelerating conditions. It is increased by increase in temperature, up to a certain limit, and by slightly hypotonic sea water and is decreased by inhibiting agents, anesthetics, KCN (Child, 1931), decrease in temperature, etc. It is also decreased by the dominance of another hydranth within a certain distance of it. Figure 115 shows the difference in length under different conditions in stems of the same diameter and, so far as could be determined, in similar physiological condition preceding experiment. Similar differences in primordium length appear in the same stem with difference in length of piece, stem-level, or with development at opposite ends of a long piece. Similar differences in scale of hydranth primordium appear in *Corymorpha*; but since perisarc is absent and the pieces change their shape by contraction and extension, measurements of primordium length are less exact.

In both these hydroids primordium determination very often occurs from both cut ends of a piece, and bipolar or multipolar forms result. In these the portion of each axis developing is determined by the scale of organization and the portion of the piece occupied by the other axis or axes. Each axis is complete from its apical end basipetally, as far as determination and development extend. In short pieces which become unipolar with hydranth at the distal end, the activation at the proximal end following section may be sufficient to shorten the gradient and hydranth primordium at the distal end, that is, to decrease scale of organization there. Under conditions resulting in difference in scale, short pieces of



FIG. 115, A, B.—Extreme differences in length of hydranth primordium in *Tubularia* pieces. A, high temperature or hypotonic sea water; B, physiological or external inhibiting factors.

the same length may give rise to forms ranging from a complete individual on a small scale (Fig. 116, *A*) through unipolar and bipolar partial forms (Fig. 116, *B-F*) to unipolar or bipolar hypostomes (Fig. 116, *G, H*).¹³ In short pieces bipolar frequency may be increased and scale of organiza-

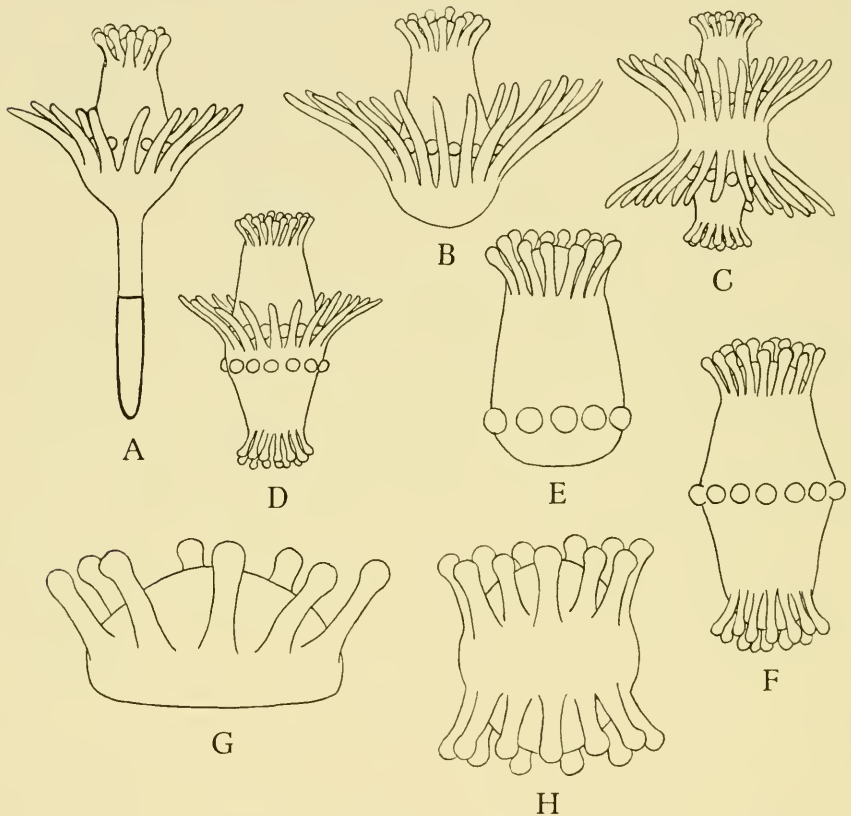


FIG. 116, *A-H*.—*Corymorpha*. Forms resulting from differences in scale of organization in pieces of the same length.

tion decreased by exposure to inhibiting agents for a short time after section and unipolar frequency, and scale of organization may be increased by differential conditioning to low concentrations of inhibiting agents or by inhibiting development at one end of the piece. This is accomplished in *Tubularia* by closing the end with paraffin or sticking it in sand, and

¹³ For earlier observations on unipolar and bipolar forms of *Tubularia* see authors cited in footnote 6, p. 36. For partial forms of *Corymorpha* see Child, 1926*b*, 1927*a, b*.

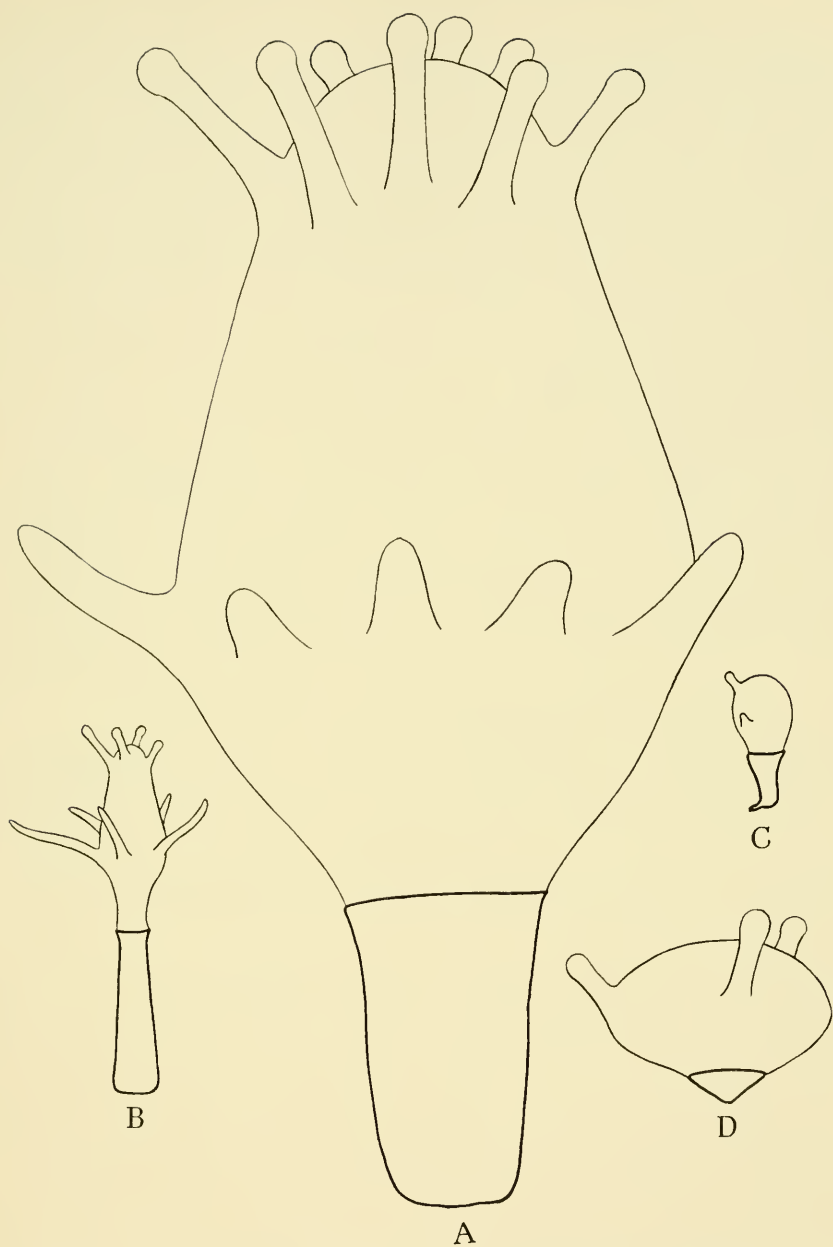


FIG. 117, A-D.—*Corymorpha*. Differences in scale of organization in aggregates of dissociated cells remaining undisturbed in contact with glass bottom of container (from Child, 1928c).

in *Corymorpha* by contact with the glass of the container or by low oxygen provided in some other way.

Differences in scale in development of aggregates of dissociated cells from *Corymorpha* stems are also extreme, not only with size of aggregate but with external conditions (Child, 1928*c*). Aggregates remaining undisturbed in contact with a glass surface usually (79 per cent) develop apical ends from the free surface and a base from the surface in contact and become either completed individuals or "mosaic" forms, consisting

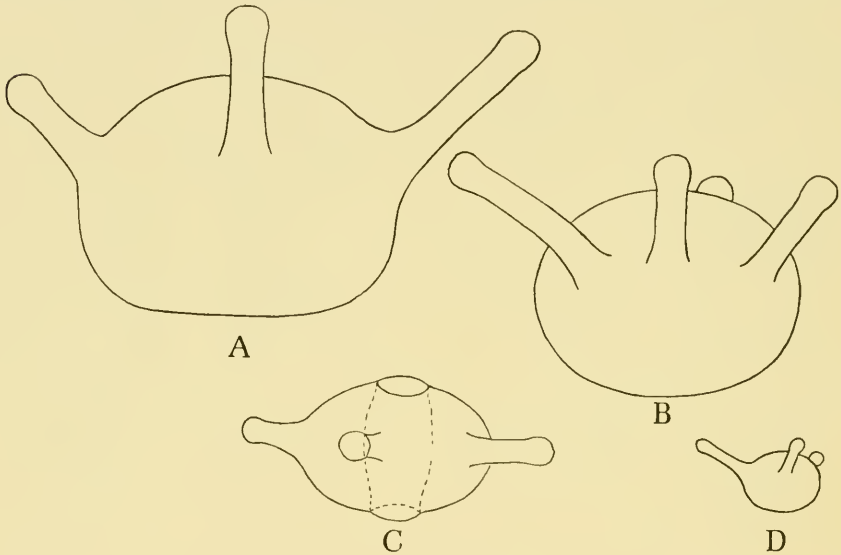


FIG. 118, A-D.—*Corymorpha*. Aggregates of dissociated cells moved about and turned over from time to time (from Child, 1928*c*).

of apical and basal parts without intervening regions. The range of difference in scale in such forms with difference in size of aggregate is indicated in Figure 117, A-D. Aggregates frequently moved about and turned over develop predominantly unipolar or bipolar apical parts of hydranths on a much larger scale (Fig. 118, A-D). There is no question as to the capacity of these stem pieces and aggregates to give rise to complete individuals. What actually develops in any particular case depends on scale of organization, as determined by physiological and external conditions.

It is evident that scale of organization determined in a piece may greatly exceed length of piece, or in cell aggregates, size of aggregate, or in bipolar and multipolar forms, length to which each axis can develop. Each gra-

dient is then only a partial gradient, and partial axial development results. It is also evident that development along each axis is determined progressively from its apical end, for development in the partial forms includes as much of the hydranth from the apical end basipetally as scale of organization and available length permit. In the naked *Corymorpha* pieces and aggregates the gradients corresponding to the partial axes can be rendered directly visible by differential dye reduction soon after section and before there is any indication of hydranth morphogenesis (pp. 97-98).

Occasionally a new axis, more or less inhibited by the dominance of a hydranth or by external conditions, determines development of only proximal parts of a hydranth—for example, a circle of proximal tentacles without manubrium, or a few proximal tentacles, or sometimes only a single tentacle at the apex of the axis. Apparently, the high end of the inhibited partial gradient in these forms is not high enough to determine the more distal parts of the hydranth. In most cases, however, either the apical part of the hydranth develops or hydranth development is completely inhibited. Similar changes in scale of organization appear in the reconstitution of other hydroids, of the scyphozoan *Haliclystus*, of the scyphistoma of *Aurelia*, and of actinians.

SCALE OF ORGANIZATION IN PLANARIAN RECONSTITUTION

A wide range in scale of organization appears in planarian reconstitution. It has long been known from work of many investigators that isolated pieces of different length may reconstitute complete individuals of different size. A long and a short piece may be of the same width, but a smaller head regenerates on the short, than on the long, piece; and the individual resulting gradually approaches normal proportions, though it may not attain them unless it is fed after reconstitution. On the other hand, pieces of the same length from different body-levels differ as regards relative size or length of parts. Size of head and distance between head and pharynx, that is, length of prepharyngeal region, decrease from anterior to posterior levels of origin of piece, or in species with fission zone, to this zone, and both increase again in the posterior zooid (pp. 44-46).

Histological studies of planarian reconstitution have led to different conclusions concerning the method of formation of the new tissue regenerating at anterior and posterior ends of a piece. Some have maintained that both the new tissue and the changes in shape and proportions of the piece result largely or wholly from migration of cells with little or no proliferation and growth, while others have observed mitoses following

section, and still others have recorded occurrence of amitosis in the new tissue.¹⁴ Those who hold that cell migration is the chief or only factor in planarian reconstitution apparently believe that some factor brings about migration not only of cells but of whole organs, such as the pharynx, until normal form and proportions are approached or attained (morphallaxis, Morgan); but the fact that these changes in form and proportion are much less rapid and less complete when the animals are not fed than in those well fed as soon as they are able to feed after reconstitution suggests that proliferation and differential growth are concerned. Growth of cells certainly takes place in the new tissue of *Dugesia*. In earlier stages of head and tail regeneration nuclei are much closer together, and there is much less cytoplasm than in fully developed parts. Whatever the role of cell migration in planarian reconstitution, other factors are certainly concerned in determining the differences in size of head and scale of organization in relation to body-level and length of piece. In pieces of equal length rate of head regeneration and length of prepharyngeal region decrease posteriorly to the fission zone (Watanabe, 1935a; Rulon, 1936a), or in species without posterior zooid, to the posterior end or as far posteriorly as head regeneration occurs. In pieces below a certain length, which differs with body-level, head regeneration is partly or completely inhibited by a stimulation from the posterior cut surface and the activation of cells following section; delay of posterior section decreases or abolishes this inhibition (pp. 181-83). Nutritive condition of the animal is also concerned; in pieces below certain lengths head regeneration is more inhibited in starved than in well-fed animals (Child, 1920a).

Form and proportions of "normal" well-fed animals apparently represent approach to, or attainment of, an equilibrium of supply and demand of material at the different physiological levels of the pattern. Reconstituting pieces differ at first from this norm according to length of piece and level of origin, and the relative requirements of parts also differ, so that some increase in size more rapidly than others and more rapidly in

¹⁴ As regards cell migration, see Stevens, 1907; Steinmann, 1926; Bandier, 1936, and citations by these authors. Bandier finds no evidence of cell division of any kind in reconstitution of a land planarian. Flexner (1898) observed mitoses following section in a planarian. In pieces of *Dugesia dorotocephala* numerous mitoses appear during the first day or two following section, both adjoining level of section and in the region in which the new pharynx develops, and scattered mitoses appear in other parts of the piece (Child, unpublished). Bardeen (1902) and P. Lang (1912, 1913a, b) believe that amitosis occurs, but Steinmann and Bandier find no amitosis. Murray (1927) observed amitosis in living parenchyma cells of *Dugesia* in tissue culture and was able to follow the stages of division from beginning to complete cytoplasmic separation. The writer has also observed these divisions in Murray's cultures.

fed than in starving animals, until the equilibrium is approached or attained. Partial individuals approach an equilibrium different from entire reconstituted individuals. In acephalic forms, for example, a posterior zooid develops, even in very short pieces, because the dominant head region is absent, and this zooid increases in length at the expense of the region anterior to it in starving acephalic forms, often becoming relatively very long. In starving intact animals different parts do not undergo reduction at the same rate. Apparently the most active, or most continuously active parts decrease least rapidly because they are able more nearly to maintain themselves at the expense of other parts than less active regions. The head decreases in size less rapidly than the body; but most of the digestive tract, in which the level of functional metabolism is undoubtedly very low in the absence of food, may disappear completely. With repeated reconstitution of starving animals scale of organization may become very small (S. J. Holmes, 1911). In short, with the progress of experimental analysis reconstitution of large individuals from long, and smaller individuals from shorter, pieces and the changes in form and proportion appear somewhat less mysterious than they have appeared to certain authors in the past. They are evidently expressions of physiological factors that can be altered and controlled experimentally. Scale of organization and morphological type of head depend upon degree of activation or inhibition of cells concerned in its formation, and this affects its rate of development and its dominance; differential growth in relation to gradient-level and available nutritive supply appears to be the essential factor in the change in form and proportion.

The distance between regenerated planarian head and pharynx, that is, scale of organization of the prepharyngeal region, can be altered experimentally in pieces from the postpharyngeal region. In pieces representing this region (*Z* of Fig. 119, *A*) the position of the pharynx reconstituting in normal environment is approximately that indicated in Figure 119, *B*, that is, somewhat anterior to the middle. Under inhibiting conditions—low concentrations of anesthetics and other toxic agents, or culture water fouled by dead planarians—head regeneration is inhibited, the head is small, and scale of organization of prepharyngeal and pharyngeal regions decreases according to degree of inhibition of the head (Fig. 119, *C*, *D*). In extreme cases no pharynx develops, and there is no reorganization of a prepharyngeal region (Child, 1911*c*, 1929*b*). Reconstitution at higher temperatures (26°–28° C.) is more rapid; heads are larger; and scale of organization of prepharyngeal and pharyngeal regions is increased. In forms

like *C* and *D* of Figure 119, most of the piece represents one or more posterior zooids, and fission often occurs anterior to the middle; that is, the dominance of the inhibited head does not extend over the whole length of the piece, often not over the anterior half.

Short planarian pieces, one-tenth or less of the body length, from levels near the head (*X* and *Y* of Fig. 119, *A*) often develop as tailless forms or forms with very small posterior outgrowths and no pharynx or mouth

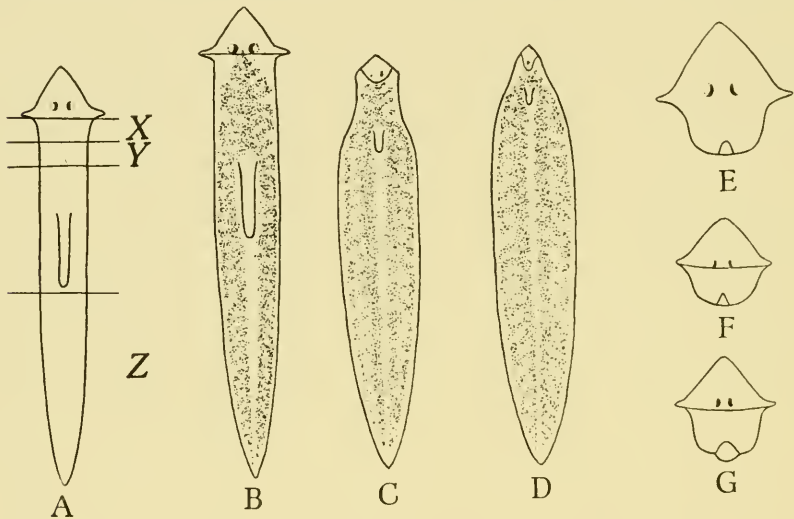


FIG. 119, *A-G*.—Scale of organization in reconstitution of *Dugesia dorotocephala*. *A*, outline, indicating body-levels of pieces *X*, *Y*, *Z*; *B*, reconstitution of piece *Z* in normal environment; *C*, *D*, decrease in scale of organization of preoral region of *Z*-pieces under inhibiting conditions. *E-G*, physiological inhibition of posterior development in pieces shorter than scale of organization; *E*, head+*X*; *F* and *G*, *X* or *Y* (*B-D*, from Child, 1929b).

(Fig. 119, *F*, *G*). Short pieces, including the original head, are also often tailless (Fig. 119, *E*).

Table 8 gives percentages of forms, either tailless or with tail inhibited, reconstituting from 1/16 pieces: *X* and *Y* (Fig. 119, *A*), 1/8 pieces; *X* + *Y*, without the original head; and *X* and *X* + *Y* with the head. According to the table, posterior development is absent or inhibited in 64 per cent of *X*-pieces, in 30 per cent of *Y*-pieces, and not at all in pieces *X* + *Y* and head+*X* + *Y*. Moreover, in *X*-pieces with regenerating head posterior development is much more inhibited (64 per cent) than in pieces with posterior ends at the same level but with the original fully developed head present (8 per cent). Apparently, in these short pieces the

anterior region activated by section or beginning regeneration is more effective in determining a scale of organization longer than the piece than is the original head. Actually, however, the effective length of the *X*-pieces for reconstitution of more posterior levels is somewhat less than the length of the piece, for the cells of the anterior part of the piece are directly concerned in head regeneration, while in head+*X*-pieces this is not the case. From isolated heads posterior ends never develop, perhaps because of lack of potency; but the table shows that at the levels *X* and *Y* length of piece, rather than presence or absence of potency, determines presence or inhibition of posterior development.

TABLE 8

ALTERATION OF SCALE OF ORGANIZATION IN RELATION TO
LENGTH OF PIECE AND BODY-LEVEL IN SHORT ANTERIOR
PIECES OF *Dugesia* (= *Euplanaria*) *dorocephala* (100 PIECES
IN EACH LOT

(Data from Rulon and Child, 1937*b*)

	Tailless	Tail Inhibited	Normal	Dead
<i>X</i>	41	23	32	4
<i>Y</i>	22	8	60	10
<i>X</i> + <i>Y</i>			100	
Head+ <i>X</i>	3	5	92	
Head+ <i>X</i> + <i>Y</i>			100	

Posterior development is also inhibited by delaying posterior section of *X*-pieces for 24 hours after anterior section, that is, by activation and initiation of head regeneration before posterior section. In all these cases of posterior inhibition the scale of polar organization determined is apparently longer than the piece. Dominance and the high gradient-levels are so effective in maintaining or determining the piece as an anterior region that not only is activation and outgrowth of cells at the posterior end largely or wholly inhibited but the piece includes no level corresponding to the pharyngeal region.

Exposure to KCN for a time after section decreases scale of organization in these pieces, so that after return to water the frequency of posterior development increases. In Table 9 posterior development is absent or inhibited in 71 per cent of the *X*-controls and in 41 per cent after KCN. In the *Y*-controls it is absent or inhibited in 31 per cent, and after KCN

in 21 per cent. The few forms listed as apolar in Table 9 are without either head or tail, and those listed as bipolar are bipolar heads. Percentages of deaths in the different lots do not differ sufficiently to affect the general result. With increase in frequency of posterior development after

TABLE 9

ALTERATION OF SCALE OF ORGANIZATION IN *Dugesia* PIECES BY EXPOSURE TO KCN
m/100,000 FOR 72 HOURS FOLLOWING SECTION (100 PIECES IN EACH LOT)
(Data from Rulon and Child, 1937b)

		Tailless	Tail Inhibited	Apolar	Bipolar	Tail and Pharynx Present	Dead
Controls in water	X.....	50	20	1		20	9
	I'.....	24	6		1	56	13
KCN m/100,000	X.....	35	5	1		42	17
	I'.....	17	1	3	2	63	14

TABLE 10

DIFFERENTIAL INHIBITION OF HEAD DEVELOPMENT IN THOSE PIECES
OF TABLE 9 WHICH DEVELOP PHARYNX AND TAIL*
(Data from Rulon and Child, 1937b)

		NUMBER OF PIECES	HEAD FORMS				
			Normal	Teratoph- thalmic	Terato- morphic	Anoph- thalmic	Acephalic
Controls in water	X.....	20	20				
	I'.....	56	48	5	1	1	1
KCN m/100,000	X.....	42	30	3	2	4	3
	I'.....	63	26			4	33

* For the characteristics of the differentially inhibited heads see pp. 177-80.

KCN there is also increase in frequency of differential inhibition of head development, as shown in Table 10. That inhibition of head development decreases scale of organization in long posterior pieces was shown by the experiments described above. Table 10 gives additional, though somewhat less direct, evidence of the same relation between head development and scale of organization in these short anterior pieces.

SCALE OF ORGANIZATION IN OTHER RECONSTITUTIONS AND
AGAMIC REPRODUCTIONS

Evidences of a very considerable range in scale of organization appear in many organisms, but only a few further examples are noted here. Pieces of *Stentor* about $1/27$ of the volume of the original individual reconstitute complete individuals (F. R. Lillie, 1896); but whether minimal size of piece capable of such reconstitution varies with level of body from which it is taken is not known. According to Morgan (1901*d*), however, the peristome is "too large" in individuals reconstituting from anterior pieces, "too small" in those from posterior pieces. These differences are evidently expressions of the longitudinal gradient shown by other methods to be present. As in planarian pieces, these differences gradually decrease, and with feeding the usual proportions may be attained.

Certain nemerteans show a very wide range of scale of organization in reconstitution. Coe regards the regenerating nemertean head region as dominant and inducing the further reorganization of the piece.¹⁵ In annelid reconstitution a wide range in scale of organization also appears. In most annelids capable of anterior reconstitution this is only in part regeneration, no more than a certain number of segments being regenerated, even though more were removed, the intervening parts being formed by reorganization of old segments into segments characteristic of more anterior levels. In some of the more transparent microdrilous oligochaetes this reorganization can be observed in the living animal, and in some other cases its occurrence has been noted, but in many studies on annelid reconstitution the question is not considered. The number of segments reorganized may vary greatly in some species, but experimental analysis of the extent of reorganization in relation to dominance of the regenerating anterior regions is largely lacking. The case of *Sabella* is of special interest because reorganization of "abdominal" into "thoracic" segments in reconstitution is directly visible, since the two regions differ morphologically, and also because the reorganization may take place in relation to a level of section before head regeneration; and in some cases reorganization of a few segments occurs anteriorly from a posterior level of section. Evidently the activation following anterior section, and sometimes that following posterior section, is sufficient to initiate the reorganization of other segments, that from the posterior section being on a small scale, as might be expected, since it opposes the polarity present. Evidently the reorgani-

¹⁵ For data bearing on the question of scale in nemertean reconstitution see Nusbaum und Oxner, 1910, 1911, 1912; Davydov, 1915; Coe, 1929, 1930, 1932, 1934*a, b*.

zation induced by the anterior activation is not specifically different from that induced anterior to a posterior section.¹⁶ In a few annelid species a large, and apparently indefinite, number of segments may regenerate anteriorly as well as posteriorly, as, for example, in *Criodrilus* (Janda, 1912a, b; Tirala, 1912). These differences in anterior reconstitution are similar to those in different planarian species, that is, in some only a head region regenerates, other parts developing by reorganization of levels posterior to the level of section, while in others parts posterior to the head also regenerate and there is little reorganization of old parts. These differences doubtless depend in both groups on physiological conditions determining rate of regeneration and rate or capacity for reorganization. In planarians it has been possible, by means of inhibiting conditions, to bring about head development almost wholly by reorganization with regeneration only at the extreme tip (see Fig. 72, *H, I*, p. 191). As in planarians, headless annelid pieces can determine development of posterior ends, but nothing anterior to the level of origin of the piece develops except in relation to a developing head region or a region activated by section and isolation from more anterior levels. Some species show great variation in scale in relation to length of piece, complete individuals developing from a single segment in certain forms, in others only from longer pieces.¹⁷ The variation in position of regenerated sex organs in reconstituted *Criodrilus* (Janda, 1912a, b) suggests variation in scale determined by differences in dominance of the developing head region.

Short annelid pieces, including the head, or from levels near the head, often fail to regenerate posterior ends, again like *Euplanaria*. This has commonly been regarded as indicating lack of potency to develop a posterior end in this anterior region, but the experiments on scale of organization in short anterior planarian pieces suggest that in the annelid, as in the planarian, scale of organization may sometimes be "too long" for the piece.

In buds, both plant and animal, the dominant apical region is the first part of the new polar axis to appear. Adventitious plant buds may originate within a single cell or involve a group of cells (see pp. 17-19), but the scale of the bud pattern increases with progress of development, and successive axial levels are progressively determined. Buds of multicellular animals apparently involve a considerable number of cells in their earliest stages, and considerable increase in size may occur before scale of organi-

¹⁶ Berrill, 1931; Berrill and Mees, 1936; Gross and Huxley, 1935.

¹⁷ Morgulis, 1907; Korschelt, 1919; Dehorne, 1932; Martin, 1933.

zation is determined. That range of dominance and scale of organization differ with conditions in buds, as elsewhere, is highly probable; but experimental analysis concerned with this problem has not been undertaken. In fission the length attained by an individual before appearance of a fission zone may vary with development of the head and with external conditions, and length of a new zooid when it first becomes distinguishable may also vary. However, the posterior zooid of a pair may not represent the entire polar axis in early stages; the axis may be determined progressively from a dominant region, as in buds (p. 332).

Questions of dominance and scale of organization in early embryonic development will be taken up in later chapters, but attention is called to a case of embryonic echinoderm reconstitution because it resembles so closely the changes in scale in hydroid and planarian reconstitution. The apical half of the sea-urchin embryo usually develops in water into an entirely ectodermal blastula-like form; but when treated with lithium, it may form entoderm and gastrulate.¹⁸ The scale of organization determined in water is apparently too large for the apical half, but with decrease of scale by lithium a complete individual develops. Short pieces of *Tubularia* and *Corymorpha* stem and short pieces of planarians show essentially identical changes under inhibiting conditions, that is, scale of organization decreases, so that a piece of a given length develops a larger part, or the whole, of the polar pattern, instead of only the apical portion. The basal half of the echinoderm embryo, however, can reconstitute an apical region and become a complete larva. This is exactly what happens in the hydroid stem pieces and in planarian and annelid reconstitution. Should we not attempt to account for all these essentially similar reconstitutions in essentially similar terms rather than by special hypotheses for the sea-urchin embryo and others for hydroids, planarians, and annelids? In many eggs and embryos scale of organization is more or less fixed at the beginning of embryonic development, or certain cytoplasmic regions must be present for complete development; these are evidently beyond the stage of simple gradient pattern.

CONCLUSION

The evidence indicates that in both plants and animals origin of a new dominant region is independent of other parts. Reconstitution of hydranth or head is not determined by other parts of an isolated piece; on the contrary, a certain degree of physiological or physical isolation from

¹⁸ See pp. 506-8; also von Ubisch, 1925*b*, 1929; Hörstadius, 1936*a*.

those parts is necessary for such reconstitution. In other words, reconstitution of a hydranth or head occurs *in spite of* the rest of the piece, not because other parts of the piece determine the replacement of parts removed. Only when activation of the cells concerned is sufficient to bring about a certain degree of physiological isolation from other parts does a hydranth or head develop from a stem piece or a postcephalic piece. The only demonstrated action of other parts on determination of a new dominant region is inhibitory. Determination of the hydranth or head represents the first steps in determination of a new polar axis. The hydranth, or its apical part, and the head, or probably the nervous tissue of the head, are primary developmental expressions of the reaction system of the species. Every stem level of *Tubularia*, *Corymorpha*, and other hydroids, every postcephalic level of *Dugesia* and many other forms, will develop as hydranth, or apical part of a hydranth, or as a head if its development is not otherwise controlled or inhibited by a dominant region or by external conditions.

A region dominant in development is an inductor or "organizer." It determines the spatial pattern and localization of parts along the axis concerned on a larger or smaller scale, according to conditions. This determination does not necessarily, probably not usually, take place all at once, but progressively from the dominant region. Scale of organization apparently depends primarily on range and effectiveness of dominance. Differences in scale represent differences in spatial localization and determination of parts. It seems evident that they depend on a factor or factors operative and effective over a certain spatial range which varies with the activity of the piece or system concerned. In view of the various other lines of evidence, the simplest assumption is that of a dynamic gradient resulting from the activation following section and from the physiological isolation from more apical or anterior regions in isolated pieces, or determined by an external differential or in some cases by localization of an activated region experimentally or otherwise without section. Such a gradient is, of course, only the initiating factor in pattern. However it is determined, its character, the changes it undergoes, and the kind of pattern and organism that results depend on the specific constitution, nuclear and cytoplasmic, of the living system in which it appears.

CHAPTER XI

RECONSTITUTIONAL PATTERNS IN RELATION TO EXPERIMENTAL CONDITIONS

RECONSTITUTIONAL development provides a wealth of material showing establishment of new developmental patterns in definite relations to experimental conditions. New patterns can be determined experimentally not only in isolated pieces of mature individuals but in eggs and early embryonic stages of some forms. From some of these experimentally determined patterns and the ways in which they are determined we can learn something about the real beginnings of development and how these beginnings can be initiated. The egg at the beginning of embryonic development usually has already an established pattern, often with considerable regional differentiation and relatively stable. Except in those cases showing capacity for reconstitution of pattern, most eggs and early embryonic stages tell us little concerning the beginnings of developmental patterns.

NEW PATTERN IN RELATION TO SECTION

In the preceding chapter it was shown that in coelenterates, planarians, nemertean, and annelids new dominant regions may be determined by the activation adjoining level of section, that they are self-differentiating, and that they may act as inductors of a new polarity. Even when this polarity is in the same direction as that of the original individual, it is a new polarity, at least as far as alteration of the pre-existing gradient and pattern extends from the dominant region. The unipolar reconstitutions of *Tubularia* and *Corymorpha* in Figure 113, *A-D* (p. 334), and Figure 116, *A, B, E, G* (p. 346), are cases in point. The bipolar forms of Figures 113 and 116 are cases in which activation at both ends of the piece was sufficient to determine a new polar pattern over a greater or less distance. *Corymorpha* pieces often give rise to multipolar forms, particularly in short pieces from levels near the basal end, from animals in poor physiological condition, under experimental inhibiting conditions, and sometimes from differential exposure. At least some of the polarities in these are localized in a definite relation to the region of section, but some may

not be. The most frequent forms of multipolarity, appearing in absence of purposely applied experimental conditions other than isolation of pieces by section, are multiplications of manubria (Fig. 120). Each manubrium is normal and complete in form from its apical region as far basipetally as it develops, and each can be shown by the various methods

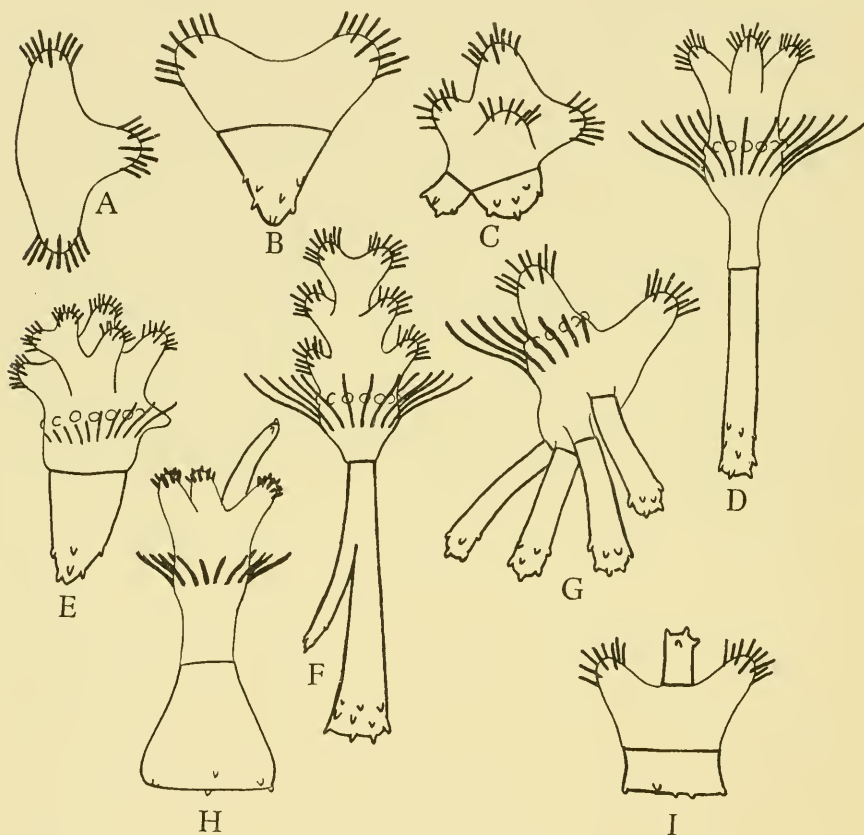


FIG. 120.—Multipolar forms of *Corymorpha* (from Child, 1926b, 1927a)

available to constitute a gradient pattern with high end apical. Forms with numerous manubria usually develop from proximal stem-levels where the diameter is greater than distally. Section at these levels often involves more crushing and laceration, and closure of the cut end is less rapid and regular than distally. The multiple manubria are apparently determined by local differences in activity of cells or cell groups on the end of the piece. There is some evidence that the longitudinal entodermal canals

just beneath the ectoderm may sometimes play a part in localizing more active regions. In pieces of smaller diameter closure of the cut end preceding development is more rapid and uniform, and a single gradient usually results.

Multiplication of basal gradients and structures is also frequent (Fig. 120, *C, F, G, H, I*), either at the end of the piece opposite that developing apical parts or elsewhere, even between multiple manubria (Fig. 120, *H, I*). As previously noted, basal axes are somewhat inhibited axes, and their development is favored by slightly inhibiting or depressing conditions. Many of the multipolar forms are mosaics of partial axes (Fig. 120, *A, B, C, I*), that is, each axis, whether manubrium, hydranth, or basal, develops from its apical region as far as its scale of organization, presence of other developing axes, and size of piece permit. These cases give further evidence that each axis develops basipetally.¹

Physical isolation of the piece from other parts, of course, plays a part in initiating reconstitution in pieces of hydroid bodies; but physiological isolation is also a factor in determining bipolar and many multipolar forms. In long pieces without the original hydranth, dominance of the distal region is insufficient to prevent development of a dominant region at the proximal end; in short pieces there is little gradient, the two ends are physiologically isolated from each other, begin development at about the same time, and neither can inhibit the other. Appearance of new dominant regions adjoining the level of section and induction of new axes in relation to them, with development of unipolar, bipolar, or multipolar forms, is very generally characteristic of coelenterates.²

In some hydroids outgrowth of tissue from the level of section precedes hydranth development, but the outgrowth represents establishment of a new gradient, as is readily demonstrated; and the high region at the tip gradually attains hydranth development if not inhibited, or grows as a stolon if it does not attain hydranth-level in consequence of dominance of other regions or inhibition by external conditions.³

¹ For other multipolar forms of *Corymorpha* see Child, 1926*b*; 1927*a, b*.

² Weimer, 1928, 1932, and many earlier papers by various authors on *Hydra*; numerous papers on many hydroid species; Child, 1933*b*, and Watanabe, 1937, the scyphozoan *Hali-clystus*; Gilchrist, 1937*c*, the scyphistoma of *Aurelia*; Child, 1903*a, b*, 1904*a*, 1908, 1909*a, b*, the actinians *Cerianthus* and *Harenactis*. See also Figs. 14, 15 (*B*), 24, 25, in chap. ii.

³ The algae *Acetabularia mediterranea* and *A. wetsteinii*, forms with a polar axis possessing a considerable degree of organization and with whorls of branches, are single uninucleate cells. They are mentioned here because of their remarkable similarity to hydroids in reconstitution. Shorter pieces may even develop as bipolar partial forms (Hämmerling, 1934, 1936). Exist-

As regards establishment of a dominant region and induction of other parts by it, reconstitution in planarians, nemerteans, and annelids does not differ essentially from that in coelenterates, except that regeneration of new tissue from the cut surface is a characteristic feature. In absence of inhibiting conditions the activation following section and isolation results in outgrowth of tissue, apparently more or less embryonic in character and undergoing differentiation. Usually the new dominance develops from this regenerating tissue at the higher end of that part of the original polar gradient present in the piece, and the tissue becomes wholly or in part a head. The length of outgrowth and the postcephalic parts developing from it differ in different forms and in planarian pieces from different body-levels of the original individual (Figs. 17-22 [pp. 43-46]). In some planarians the outgrowth may be greatly decreased by inhibiting conditions, so that the head develops in large part by reorganization posterior to the level of section (Fig. 72, *H* [p. 191]; Fig. 119, *D* [p. 352]). Under these conditions planarian head reconstitution approaches the type of reconstitution of hydranth in *Tubularia* and *Corymorpha*. Anterior outgrowth of new tissue and head development are increasingly inhibited with decrease in length of piece in several planarian species; and in pieces below a certain length, which differs in a definite way with level of origin of piece, size and physiological condition of animal, and external conditions, there is complete inhibition of head development, but development of a posterior end is possible at the same level under the same conditions (pp. 180-90). In absence of the tissue outgrowth from which head develops, little or no reorganization of body-levels anterior to level of origin of the piece takes place, but all parts posterior to that level can still develop. For example, pieces from the prepharyngeal region, cut short

ence and movement in opposite directions of two formative substances are postulated by Hämmerling, but it seems evident that dominance and a gradient are present along the axis and that determination of new dominances and gradients by activation following section are concerned in the reconstitution of these plants. Moreover, the postulated movement of formative substances in opposite directions from the nucleus, which is situated near the basal end, in unipolar reconstitution of pieces, seems to require the presence of an axial differential of some sort. The fact that nonnucleated pieces possess considerable capacity for reconstitution appears to support this view. Whatever the situation as regards movement of substances, orderly movement in opposite directions seems to require presence of a pattern of some sort, and the data of reconstitution of these algae suggest that that pattern is a gradient. Substances from the nucleus may, of course, play an important part in determining physiological condition and the character of later differentiation of parts, but how they can determine the general spatial pattern and axiate order and the localization of secondary axes as lateral branches does not appear.

enough to be acephalic (*I* of Fig. 121, *A*), develop pharynx and mouth and all parts posterior to the mouth (Fig. 121, *B*); but acephalic pieces from a level just posterior to the mouth (*II* of Fig. 121, *A*) do not develop mouth, pharynx, or prepharyngeal region (Fig. 121, *C*). However, if there is even rudimentary head development (anophthalmic [p. 179]) in pieces from postoral levels, the anterior part of the piece reorganizes into a prepharyngeal, pharyngeal, and oral region (Fig. 121, *D*). Moreover, such forms, even though head development never goes beyond this stage, are distinctly more like normal animals in motor behavior than are acephalic forms.

Many investigators have recorded development of bipolar forms from short planarian pieces.⁴ Such forms may consist merely of heads alone or of heads and more or less of the anterior post-cephalic region, and sometimes a pharynx develops in one or both axes. Occasionally also one or two posterior ends develop from lateral regions, usually after more or less elongation in consequence of the opposed locomotor activity of the two heads (Fig. 122). Both heads may be normal; or one, usually the posterior, may be more or less differentially inhibited (Fig. 122, *E*). Occasionally both head and posterior end develop from a posterior cut surface,

particularly if it is somewhat oblique (Fig. 122, *G*; also Fig. 25, *D* [p. 52]). Bipolar forms may apparently develop from any body-level in sufficiently short pieces, but in the species with a definite postoral fission zone they seem to appear most frequently from the region of the fission zone, with pieces of given length. In this region there is a slight rise in gradient-level from the posterior end of the anterior zooid to the anterior end of

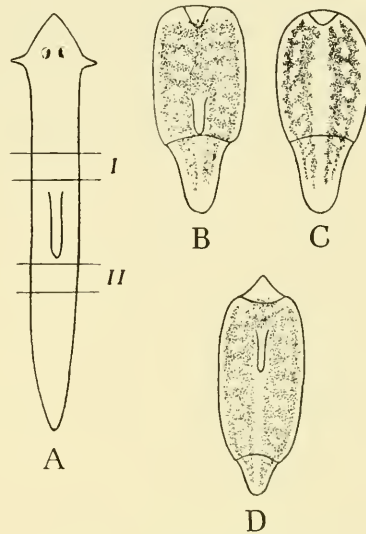


FIG. 121, *A-D*.—Development of acephalic planarian forms from different body-levels. *A*, outline indicating levels of sections *I* and *II*; *B*, prepharyngeal acephalic form from level *I*, pharynx and mouth reconstituted; *C*, postpharyngeal acephalic form from level *II*, does not reconstitute pharynx or prepharyngeal region; *D*, postpharyngeal piece with rudimentary anophthalmic head, reconstitutes pharynx, mouth, and prepharyngeal region (*B-D*, from Child, 1911*c*, 1929*b*).

⁴ See, e.g., Morgan, 1898, 1900*a*, 1904*a*, *b*; Bardeen, 1902, 1903; Child, 1911*b*; P. Lang, 1913*a*; Rustia, 1925; Lus, 1926; and other authors.

the posterior zooid; consequently, there is little or no gradient difference at the two ends of a short piece from this region, and neither is dominant. A planarian head sectioned transversely at the level of the cephalic ganglia sometimes becomes bipolar by development of a second head opposed in orientation from the cut surface (Fig. 122, *F*).

Bipolarity in short planarian pieces from other levels than the fission zone can be greatly increased by differential inhibition with various agents (Rustia, 1925). According to the gradient concept, the two cut ends of such pieces differ but little because only a small fraction of the

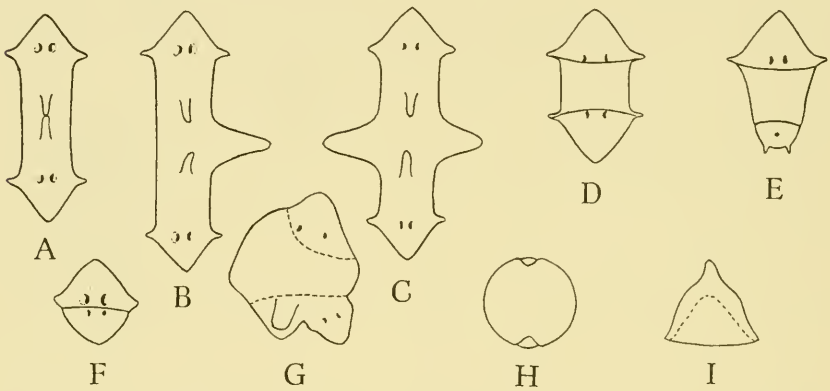


FIG. 122, A-I.—Bipolar and apolar forms from short planarian pieces. A-F, *Dugesia*; G, *Cercyrya papillosa*, a marine triclad (after Lus, 1926); H, I, apolar forms.

polar gradient is included between them. Differential inhibition decreases this difference still further, so that after return to water the two ends are so nearly alike that neither can dominate the other; consequently, the activation at each end is independent of that at the other, and the new tissue develops into a head and determines a new polarity as far as length of piece, scale of organization, and presence of the other polarity permit.

Under the usual conditions and under most experimental conditions thus far employed, bipolar heads do not develop on pieces $1/4$ or more of the body length of large, mature planarians; but in somewhat hypertonic modified Ringer solutions bipolar heads frequently appear on such pieces (Child, unpublished). Since loss of motor co-ordination and more or less complete paralysis occur in these solutions but regeneration is not much inhibited, the decrease in dominance results in physiological isolation of the posterior cut end to a degree permitting the activated cells there to develop a head instead of a posterior end.

Extremely short planarian pieces often appear to be completely apolar.

By contraction of the cut ends they become circular in dorsal or ventral aspect, new tissue merely fills in the contracted cut surfaces without outgrowth, and definitely directed locomotion is absent (Fig. 122, *H*). In consequence of contraction of the cut surfaces the longitudinal muscles function much like the circular muscles in a medusa, their contraction decreasing the circular diameter of the piece. Since the ventral muscles are stronger, the central regions are forced dorsally when they contract, and in time a permanent change in form with more or less elongated dorsal outgrowth results (Fig. 122, *I*). These forms finally appear completely radial in form and motor activity. It is suggested that outgrowth of tissue is absent in these pieces because the whole piece is involved equally or almost equally in the changes following section; that is, the pieces are so short that a differential between regions adjoining the cut surfaces and other parts of the piece is practically absent, the whole piece adjoins a cut surface, and consequently there is no differential developmental behavior of any kind except that finally resulting from the radial muscular activity.

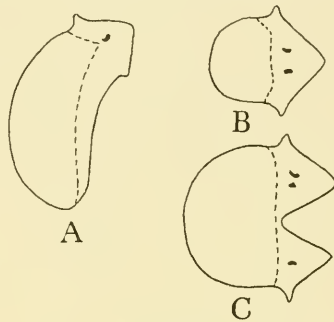


FIG. 123.—Reconstitution of short lateral planarian pieces (from Beyer and Child, 1930).

Bipolar posterior ends may develop from short posterior pieces of certain triclad species that do not undergo fission (Morgan, 1904*a*; Lus, 1926). In these cases activation is not sufficient at either level of section to determine dominance and independence of other parts, and reconstitution does not involve independent origin of a new pattern but development of a subordinate part determined by other parts of the piece. Undoubtedly differences in physiological condition at different body-levels may be concerned in determining whether bipolar heads or bipolar tails develop, but lack of potency for head or tail development is not necessarily involved. Bipolar heads or tails may be determined by a difference in relation between the activated cells and other parts: if they become independent, heads develop; if they remain subordinate, tails develop.

Planarian pieces from the region lateral to the ventral nerve cords show increase in frequency of head regeneration from the longitudinal cut surface on the median side of the piece rather than from the anterior transverse surface, with decreasing length of piece. The head may regenerate on the angle between the two cut surfaces (Fig. 123, *A*); or in very short pieces, so strongly contracted that transverse and longitudinal cut

surfaces are indistinguishable, one or two heads may develop on the whole surface of section (Fig. 123, *B*, *C*). Apparently, with decrease in the

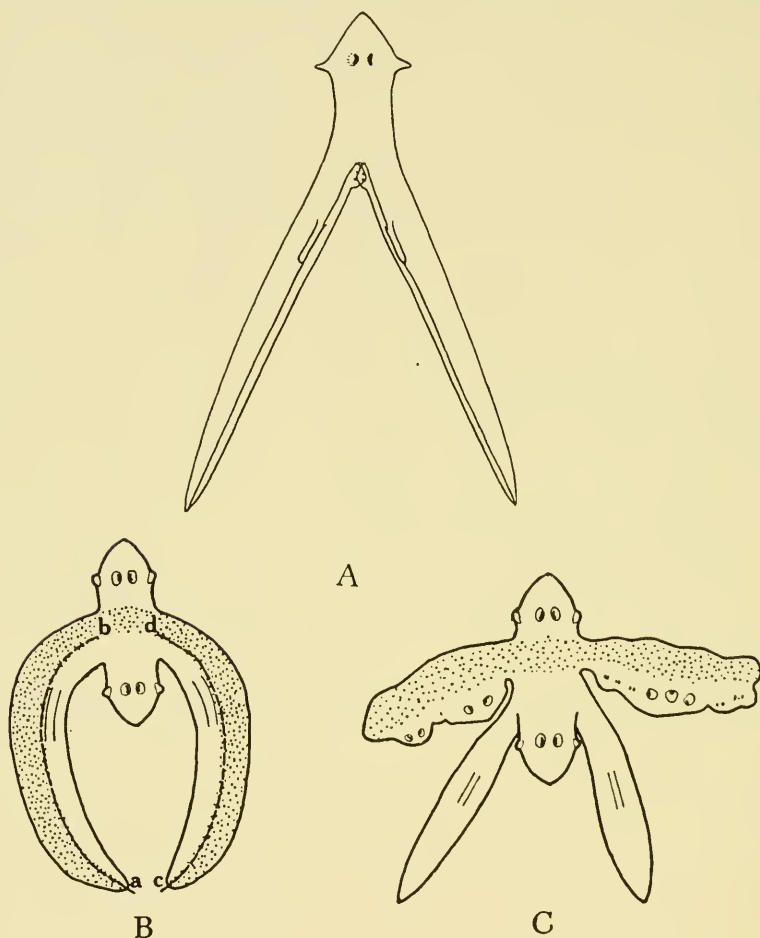


FIG. 124. *A-C*.—Planarian forms with multiple heads after partial posterior longitudinal splitting. *A*, two heads from anterior regions of sides of split; *B*, *duplicitas cruciata*; *C*, split twice, the second split dividing each of the parts separated by the first; cruciate after first split, multiple heads from longitudinal surfaces of section of lateral posterior regions after second split (*B*, *C*, from Silber and Hamburger, 1939).

anteroposterior differential, as length of piece decreases, the mediolateral differential plays an increasing part in localization of the head.⁵

⁵ Child, 1915c, p. 164; Olmsted, 1918; Beyer and Child, 1930.

Certain cases of bipolarity and multipolarity in planarians are of special interest. One or two heads occasionally appear at the anterior end of a longitudinal split extending over most of the body length from the posterior end (Fig. 124, *A*, *B*).⁶ Probably a certain degree of physiological isolation is concerned in the development of these heads; such isolation results when there is little direct nervous connection with more anterior regions. In these cases the longitudinal split is between the nerve cords; consequently, only cut transverse commissures can be present at the surfaces of section. If they are present, they may play a part in localizing head development. As regards the new tissue developing on the longitudinal cut surfaces, the heads are anterior, perhaps because gradient-level and reactivity are higher there, though head development may occur at more posterior levels of a longitudinal split, as will appear below.

Cruciate forms with a single head at the anterior end of the longitudinal split (Fig. 124, *B*) and a variety of multipolar forms resulting from the same operation have been described for *Dugesia tigrina* by Silber and Hamburger (1939). One of these, resulting from a second longitudinal split of each part separated by the first split, is shown in Figure 124, *C*. This form became cruciate after the first split; but after the second, heads regenerate from the longitudinal cut surfaces of the two lateral posterior ends. Three distinct heads and five other pairs of eyespots in early stages are present on these surfaces. The ventrodorsality of these heads coincides with that of the body, and their bilateral form is evidently due to the fact that each one represents a localized region of outgrowth with a ventrodorsal pattern. The nervous system in these lateral posterior ends must be undergoing or have undergone extensive reorganization; but whether parts of it are concerned in localizing the outgrowths which become heads does not appear. In some species of planarians more readily than in others a head may regenerate from a lateral region partly separated from the body by an oblique section extending posteriorly and with its anterior end removed to provide an anterior cut surface; from this the head develops. Provided the longitudinal separation persists, an indefinite number of heads along the sides of the original body, with more or less induced reorganization posterior to each, may be produced. This is essentially a variation of the experiment of Figure 135, *A* (p. 400).

As regards establishment of new dominant regions, determination of

⁶ Van Duyne, 1896; Morgan, 1900a; Goetsch, 1921, 1922, 1928; Keil, 1924; Beissenhirtz, 1928; Silber and Hamburger, 1939.



new gradients and induction of reorganization, annelid, and planarian reconstitution do not differ greatly. It was noted earlier that in most annelids only a certain number of segments, characteristic for the species, regenerates anteriorly after removal of a larger number, the other remaining segments removed being formed by reorganization of old segments induced by the new dominant region. A few annelids regenerate a large number of segments anteriorly.⁷ Attention is called again to the case of *Sabella* in which "abdominal" and "thoracic" segments differ morphologically. Regeneration from anterior ends at abdominal levels induces reorganization of some abdominal into thoracic segments. This reorganization may also occur after section and before head regeneration. Also, in some cases reorganization of segments anterior to a posterior section in the abdominal region takes place; this represents an approach to bipolarity. Apparently the activation following section and isolation is sufficient to bring about induction without actual head regeneration.⁸ According to Berrill and Mees, visible light increases greatly the number of segments reorganized; in some way it apparently makes the dominance established following section effective over a greater distance. The authors suggest that the effect of light involves a photochemical reaction and release of electrical energy rather than diffusion of a chemical substance.

Regeneration of posterior ends in planarians and annelids involves establishment of a growth gradient opposite in direction to the primary polar gradient. In short planarian pieces and apparently also in *Lumbriculus* the posterior activation may inhibit head regeneration (pp. 183, 406). After completion of posterior regeneration in planarians this gradient persists chiefly at the extreme posterior end and is associated with growth in length; but in the species with one or more posterior zooids these grow in length more rapidly than the anterior zooid. In the annelids the posterior gradient becomes a gradient of segment development and growth, with high end posterior, but anterior to the anal segment, and may persist throughout life in some species or disappear later in others.

Bipolar partial forms with heads at both ends develop in pieces from anterior regions, and bipolar posterior forms in pieces from posterior re-

⁷ For numbers of segments regenerated anteriorly and reorganization of old segments see Ivanov, 1908; Hyman, 1916a, and citations; also Morgulis, 1907; Korschelt, 1919; Martin, 1933; also Hyman, 1940, "Aspects of regeneration in annelids," *Amer Nat.*, 74.

⁸ Berrill, 1931; Berrill and Mees, 1936a, b; Gross and Huxley, 1935. In his earlier paper Berrill regards the regenerated head as organizer, but it seems evident from the later papers that the activation following section, rather than the head itself, is the inducing factor.

gions, of various annelids.⁹ As in planarians, level of origin of piece, gradient differential in it, and intensity of activation at the cut ends may be the factors determining at least some of these bipolar forms rather than specific differences in potency for head or posterior development at different levels.

Pieces of the ascidian *Clavellina* from the "esophageal" region may reconstitute complete individuals or become bipolar partial forms by development of siphons, branchial chamber, and other distal parts at both ends (Brien, 1934). Stolons attached to the body develop new individuals at their distal ends; stolon pieces isolated by section reconstitute individuals from their proximal ends, probably because activation of cells at this end by section and isolation is sufficiently intense to establish dominance and a gradient which inhibits the less rapid bud development at the distal end. The bipolarity of *Clavellina* pieces is certainly not due to restriction of potency, for experiment has shown that proximal, as well as distal parts, can develop from the proximal ends of pieces like those that become bipolar, and there is evidently no limitation of potency in the stolon. Brien's interpretation of his experimental data is in terms of gradient relations.

The relative roles of reorganization of old parts and regeneration of new tissue vary in different species and groups among the lower invertebrates; in some planarians they differ in definite ways with body-level of origin of piece and with experimental conditions. In the higher invertebrates and in vertebrates, reconstitution in mature individuals is chiefly or wholly by regeneration and is, in general, limited to regeneration of subordinate parts, such as appendages, or in higher vertebrates almost entirely to regeneration of a particular tissue by cells of that tissue.

Regeneration of legs of arthropods and amphibians evidently involves origin of a new longitudinal axiate pattern. The regenerate is at first bud-like in appearance; but, as regards gradient pattern, little is known. In the regenerating legs of certain insect nymphs (Agrionidae) the tarsal claws usually become distinguishable as definite, localized outgrowths in very early stages, long before muscle attachments and articulations appear; that is, the first visible evidence of structural differentiation is at the tip of the regenerate. Development of muscles, tendons, and articulations, however, generally progresses from the proximal region distally; and when regeneration begins in later nymph stages muscles and articulations are often absent from the distal region, or the whole leg may have

⁹ Morgan, 1902; Korschelt, 1904, 1927-31; Gates, 1927; von Haffner, 1931.

small tarsal claws but no muscles or articulations. These data suggest an ectodermal gradient with high end at the tip, at least in earlier stages, and mesodermal development progressing from the proximal region distally (Child and Young, 1903).

In the regenerating amphibian leg skeleton and skin develop, even though they have been more or less completely removed from the proximal stump.¹⁰ The longitudinal half of the leg of *Triton* does not regenerate the half removed; but the distal part, regenerated from a transverse surface of section of such a half-leg, may be complete and normal as regards polarity and asymmetry.¹¹ The distal portion of a leg may regenerate from the proximal cut surface of part of a leg implanted by its distal cut end (p. 390). Apparently the primary polar pattern of the regenerate is not determined by the part from which it develops but originates in the budlike outgrowth. However, the mitotic index in earlier stages of regeneration is highest near the base and shifts distally as regeneration progresses (Litwiller, 1939). Also, structural differentiation, practically all mesodermal, progresses from the proximal region distally. Except for these data, nothing is known concerning gradient pattern in the regenerating or the embryonic amphibian leg. Undoubtedly there is a radial gradient system, decreasing from a center in the early stages; but whether the high central region becomes the distal tip or remains proximal or the high region becomes distal in the ectoderm and proximal in the mesoderm, or whether the gradient pattern changes in the course of original development and regeneration, remains to be determined. Early stages of appendages in the chick embryo show dye reduction decreasing radially from a center, but information concerning dye reduction in earlier stages of the amphibian leg is lacking, partly because of pigmentation and the difficulty of staining with the oxidized dyes.

In all these cases of reconstitutive development of a new axis in relation to section it originates either as a direct alteration of a pre-existing axis by a new dominance and gradient, as in *Tubularia* and *Corymorpha*, or as an outgrowth of new tissue much like a bud in that developmental activity at first decreases more or less radially from a center and the radial decrement becomes longitudinal by differential growth. In hydroids the radial symmetry of the new axis may originate anew in the bud or may develop in relation to the radial pattern of a stem piece. For ex-

¹⁰ Weiss, 1925, 1927a; Bischler, 1926; Liosner, Woronzowa, und Kusmina, 1936; and citations by these authors.

¹¹ Weiss, 1925c, 1926b. See also Gräper, 1926a, b.

ample, in *Corymorpha* pieces or aggregates radial pattern may develop quite independently of the original radial pattern, but in stem pieces isolated by transverse section the longitudinal entodermal canals of the stem which are parts of the original radial pattern are factors in localizing tentacle development.

SOME SPECIALLY SIGNIFICANT CASES OF NEW PATTERNS
IN RELATION TO SECTION

Under natural conditions the dominance of the apical hydranth of *Corymorpha* prevents development of lateral hydranth buds, and the hydranth-stem axis remains permanently unbranched. A simple lateral transverse section part way through the *Corymorpha* stem closes in a few hours but may become the locus of development of a new hydranth and stem, provided the original hydranth is removed. In experiments involving several hundred animals the frequency of hydranth development from a simple incision, with removal of the apical hydranth at the same time, was 56.7 per cent at a level near the distal end, 26.7 per cent at a middle level, zero at a proximal level; with the apical hydranth present and in good condition it was zero at all levels (Child, 1927c, 1929a, 1932a). However, even a small lateral incision, with edges lacerated by repeated small cuts so that healing does not occur so rapidly and smoothly, becomes a new hydranth-stem axis after healing in almost 100 per cent of the cases if the original hydranth is removed (Fig. 125, A-C). Later an outgrowth, and in some cases a differentiated basal region, may develop from the opposite side of the original stem (Fig. 125, D). In these forms, as in other *Corymorpha* reconstitutions, hydranth development is not from a cut surface but from a region activated by the lacerated incision; development begins only after the closure of the incision. The activated region determines a new gradient, at first radial, as in buds generally, and becoming longitudinal by differential growth, and acts as an inductor, sometimes being effective across the original stem in inducing development of a basal region where there is no incision or other injury. Under slightly inhibiting conditions the lacerated incision may give rise to a basal region with perisarc and holdfast buds instead of a hydranth (Fig. 125, E).

New axes have been localized in a somewhat similar manner in the elongated actinian *Harenactis attenuata* (Child, 1909c, 1910c). After removal of muscles and mesenteries from the inside of short pieces isolated by transverse sections from levels proximal to the esophagus contraction brings distal and proximal cut surfaces of the body wall of the piece to-

gether about the whole circumference, and union results, forming a closed ring like the inner tube of an automobile tire. Usually these rings undergo a rather remarkable orientation which brings the line of union of distal and proximal ends onto the upper surface as the ring lies. Along or adjoining the band of new tissue uniting distal and proximal ends tentacles and tentacle groups, varying in number and arrangement of tentacles, develop from either distal or proximal side of the union or from both, and in some cases there is approach to development of an actinian axis

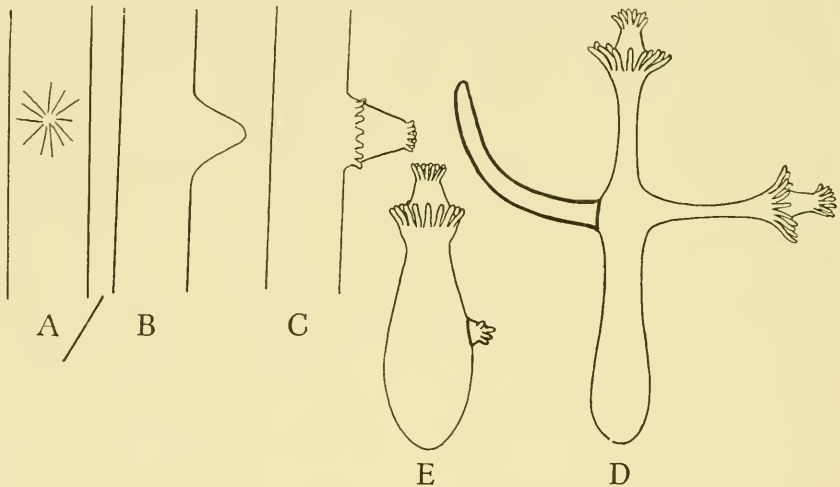


FIG. 125, A-E.—Determination of new axis by a lacerated lateral incision in *Corymorpha*. A-C, incision, later outgrowth and early hydranth; D, induction by the new dominance of a basal region from the opposite side of the original stem; E, development of a basal end with perisarc and stolon buds from a lacerated incision under slightly inhibiting conditions (from Child, 1927c).

(Fig. 126, A-C). Some of the tentacle groups are bilateral, others radial, and still others irregular. Examination shows that the tentacles of these groups are localized between the regenerating mesenteries and that the form of the group depends on the positions of the mesenteries on the two sides of the line of union and the directions in which particular mesenteries happen to grow toward the line of union. When there is definite axiate outgrowth, as in Figure 126, C, some of the mesenteries reconstitute with the outgrowth and determine the number of tentacles at its apical end. The groups appear to be localized where reconstitucional activity is greater.

After local laceration of distal and proximal cut ends of the body wall

by a number of longitudinal incisions close together and so placed that, when contraction and distal-proximal union occur, distal and proximal lacerations will come together, much more new tissue is formed in the course of union, and in some cases a complete new disk with normal number of tentacles and with mouth and esophagus develops from the lacerated region, and development of more or less of the body follows

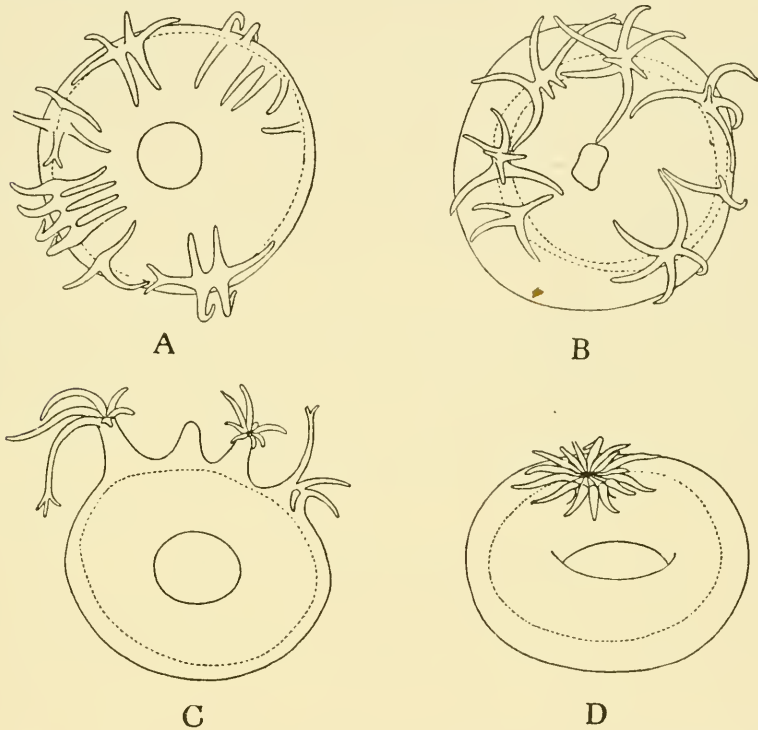


FIG. 126, A-D.—Development of tentacle groups and new axiate patterns in “rings” of *Harenactis attenuata*; further explanation in text (from Child, 1909c, 1910c).

(Fig. 126, D). The activation following laceration and union has determined a bud which becomes an entirely new polar axis and gives rise to the distal parts of a normal individual. Here the old mesenteries are not concerned, but the normal number of new mesenteries of a young individual develops. It seems beyond question that the larger amount of new tissue developing and probably a more intense and extensive activation following the laceration are factors in determining the entirely new axis of completely normal character as far proximally as it develops. Evi-

dently the pattern of the new disk of Figure 126, *D*, is not determined by the original pattern but has arisen, like other bud patterns, from a region of local activation involving both distal and proximal levels of the piece. The tentacle-bearing outgrowths of Figure 126, *C*, apparently represent less intense activation and less complete dominance; in them mouth and esophagus do not appear. The significance of these cases is twofold: they provide further evidence to show how completely independent of the old pattern the new pattern may be, and must be for development of a new polarity; and they agree with other data in indicating that differences in intensity and probably in extent of activation may give different morphological results. They do not differ essentially from cases of reconstitution of new dominant regions but are merely rather striking examples of establishment of new dominance by localized activation, with more or less induction resulting, in *Corymorpha*, even across a pre-existing pattern (Fig. 125, *D*).

Bud formation in *Hydra*, resulting from local cautery (Tepliakova, 1937), and determination of head development in a planarian by local cautery, laceration, and application of certain chemical substances, as reported by Goldsmith (1932, 1933, 1934, 1937, 1940), are similar cases in which the local injury brings about sufficient activation to initiate development of a new dominant region and polar pattern.

QUESTIONS OF PATTERN AND ITS ALTERATIONS IN EARLY EMBRYONIC RECONSTITUTIONS OF ISOLATED PARTS

Data of early embryonic reconstitutions are discussed in the following chapters. Here it is desired to call attention to certain questions concerning effects of section and isolation on egg and embryonic pattern, suggested by the reconstitutions of isolated pieces of adult animals. First, it may be noted that in isolated parts of eggs and isolated blastomeres or blastomere groups there is no evidence of regeneration in the strict sense, no outgrowth in relation to a surface of section. Whatever reconstitution occurs is reorganization within the part. What happens to the pattern present in the part when it reconstitutes a whole? We know even less about what happens in these cases than we do concerning reconstitution in an isolated piece of an adult hydroid or planarian, but at present there seems to be no good reason for regarding embryonic and adult reconstitution as essentially different. Gradient patterns have been demonstrated in many eggs and embryos. These are unquestionably altered in one way or another in reconstitution, as they are in adult reconstitution. Direc-

tion of polar pattern is usually not altered in the early embryonic reconstitutions, as far as known; but there are cases in which it cannot be determined whether it is altered or not. There is often considerable change in scale of organization when half an egg or an isolated blastomere becomes a complete individual. What happens to the pattern in these cases? Various interpretations have been offered, in terms of hypothetical formative substances, of substance gradients and their changes, etc. But if substances do change their positions in definite and orderly manner in these cases, these changes must be determined by a pattern of some sort. What is the character of that pattern? Does the reconstitution of an apical region on the basal half of an eight- or sixteen-cell sea-urchin embryo differ fundamentally in any way from reconstitution of a hydranth in a piece of *Tubularia* or *Corymorpha* or a head on a planarian piece? Does not failure of the apical halves of the same stages to develop mesenchyme and entoderm present the same problems as failure of isolated hydranths or heads to reconstitute more proximal or posterior parts? With a large scale of organization a short hydroid or planarian piece may develop only the apical or anterior part of an individual. With decrease in scale it may become an entire individual. The same is true for the apical half of the sea-urchin embryo. Under natural conditions it forms only the more apical portions of an individual. With experimental decrease in scale it may become a complete individual (p. 357).

Ventrodorsality and dorsiventrality may be reconstituted in definite relation to the pattern already present, though in many cases of blastomere reconstitution it is not known whether or not this is the case. Suppose, for example, we isolate the blastomeres of the two-cell stage of a form in which ventrodorsal pattern of some sort is present at this stage but the first cleavage plane has no definite relation to it. What happens when either or both blastomeres develop as entire individuals? Is the entire ventrodorsal pattern present on one side and reconstituted on the other, or is only a part of it present and the rest reconstituted, or is a new ventrodorsality established on a smaller scale? If ventrodorsality is primarily a gradient pattern, any one of these effects is possible. If ventrodorsality or dorsiventrality has developed beyond the simple gradient stage, reconstitution of a complete individual may be possible only with isolations at or near the median plane.

Embryonic reconstitution is narrowly limited or practically absent in some forms, apparently in consequence of highly stable regional differences in the cytoplasm. Similar limitations appear in the adults of various

species. It has often been noted, sometimes as something remarkable and difficult to account for, that embryonic stages of certain forms appear almost completely incapable of reconstitution, while adults of the same species have high reconstitutive capacity. This is the case in many annelids. The only difficulty here is the failure to recognize that the egg may have a higher or more stable regional differentiation than different levels of the adult body and that much of the egg differentiation may be lost during development. In the annelid the embryonic differentiations give rise mostly to larval parts and to head regions; it has never been shown that the cells from which postcephalic regions develop possess any such differentiation. In fact, there is evidence from their reconstitution that they do not.

With the progress of experimental investigation and analysis it appears increasingly evident that essential features of developmental pattern in many eggs and early embryonic stages are fundamentally similar to features of pattern in adults of many of the simpler organisms, as far as spatial order and relations are concerned. If this is true, reconstitutive development in adult material, being usually more accessible to experimental analysis than that in parts of eggs and embryos and bringing us nearer the beginnings of developmental pattern than the beginning of embryonic development in many forms, should be regarded as an invaluable aid in throwing light on problems of embryonic development and reconstitution.

A few data concerning modifications of embryonic pattern in relation to section and isolation and involving points of special interest are briefly discussed. Alteration by section of the polar pattern in the egg of the sea urchin *Lytechinus variegatus* has been reported. Using position of polar bodies and micropyle for identification of the original polar axis, it was found that "animal" and "vegetal" halves, meridional halves, and halves resulting from section in random directions of unfertilized eggs may, after fertilization, reconstitute into wholes. Pieces down to $1/20$ of the total egg volume, irrespective of the region concerned, may become normal blastulae with primary mesenchyme; micromere formation is not necessary for formation of primary mesenchyme. Section of unfertilized eggs of *Strongylocentrotus purpuratus*, *S. franciscanus*, and of the starfish *Patiria miniata* is followed after fertilization by first and second cleavages in a plane vertical to the plane of section; and when plane of section can be identified at the stage of gastrulation, invagination occurs on the surface of section. Moreover, in *Lytechinus* micromeres form on the surface

of section.¹² These results give little evidence of any pattern of localized substances or regional cytoplasmic differentiation. Even polarity is altered by section, according to these authors, the surface of section becoming the basal region. With isolated pieces of unfertilized and fertilized eggs of *Arbacia* somewhat different results are obtained by Harnly (1926). Localization of micromere material between nucleus and center of egg is indicated, and micromeres do not form on the surface of section. According to Plough (1927, 1929), development of isolated $1/2$ blastomeres of *Arbacia*, *Echinarchnius*, *Echinus*, and *Paracentrotus* indicates that micromere material is localized at the time of first cleavage; but neither this localization nor that postulated by Harnly agrees with the results of Taylor, Tennent, and Whitaker. According to results obtained with other species, polarity is not altered by section.¹³

These various results cannot, at present, be brought into agreement, and it is not likely that all of them represent species differences. Perhaps, however, if the polar pattern of the sea-urchin egg is primarily a quantitative dynamic gradient system rather than a pattern of localized specific substances, the different results may prove less difficult to reconcile. The high temperature of the water in which *Lytechinus* lives (Tortugas) may conceivably account for the apparent susceptibility of the polar pattern to section. As regards development of micromeres from the surface of section in *Lytechinus*, it has been found that in *Strongylocentrotus* and *Dendraster* the micromeres appear at the low end of the primary polar dye-reduction and susceptibility gradients. They, together with prospective entoderm, apparently undergo an activation preceding gastrulation (pp. 134-40). In the light of these data it appears possible that micromeres might form on the surface of section in consequence of a depression resulting from the injury to the cell.

Sectioning sea-urchin eggs or early embryos in or near the frontal plane results in reversal of ventrodorsality in the dorsal but not in the ventral portion. Ligaturing in a meridional plane may determine ventrodorsality vertical to the plane of ligature. With ligature tightly drawn, two opposed ventrodorsalities result, dorsal sides of both being toward the ligature.¹⁴ If the ventral side is the high end of the ventrodorsal gradient, as it appears to be, there is apparently depression or inhibition in the plane

¹² Taylor and Tennent, 1926; Taylor, Tennent, and Whitaker, 1926; Tennent, Taylor, and Whitaker, 1929.

¹³ Hörstadius, 1936a, b, 1937; Hörstadius und Wolsky, 1936.

¹⁴ Hörstadius, 1936a, b, 1937, 1938; Hörstadius und Wolsky, 1936.

of isolation with reversal of ventrodorsality in the dorsal part, perhaps in consequence of physiological isolation (p. 306). But only by further experiment can we hope to discover what really happens in these cases. It seems evident, however, that ventrodorsality is not fixedly determined in these early stages but can be altered, as was maintained by Boveri (1902).

INDUCTION OF RECONSTITUTION BY IMPLANTS IN MATURE ANIMALS

It was shown by Browne (1909) and confirmed by later workers that even small pieces of *Hydra* from the region about the base of the tentacles, implanted laterally, can persist and determine new apical regions and polarities, largely from host tissue; that is, the implant is dominant and acts as an inductor. Similar small pieces from other regions of the body are incorporated in the body wall or resorbed; but large pieces may persist, reconstitute an apical region, and induce. Pieces of stalk, especially those bearing the foot, tend to persist and grow without modification. It is perhaps of interest in this connection to recall that dye reduction in low oxygen occurs early in the foot of the fully developed animal and that, if the stalk contracts actively, it reduces more rapidly than the body (p. 101).

Autoplastic or homoplastic implantation of very small fragments, even $1/4$ sectors of very short transverse stem pieces of the hydroid *Corymorpha* in small lateral incisions in the stem, may determine new hydranth-stem axes, the graft itself forming only more or less of the apical region of the hydranth and inducing other parts from the host tissue (Fig. 127, A-C). Development of the new axis sometimes occurs when the apical hydranth is present; but its frequency ranges from 35 to 75 per cent higher, according to level of origin and of implantation, when the apical hydranth is removed. Grafts from a distal donor-level, that is, from a higher gradient-level, are more effective in inducing a new axis than those from proximal levels, whether level of implantation is distal, middle, or proximal (Child, 1929a, 1932a). In series of comparable experiments with hosts and grafts and donors of approximately the same length and those of each lot collected at the same time, pieces from a distal donor-level gave a total frequency of new axes at distal, middle, and proximal host-levels of 16.6 per cent with the apical hydranth present and in good condition; pieces from a proximal donor-level, only 3 per cent with apical hydranth present. With removal of the apical hydranth at the time of implantation percentages were, respectively, 73.3 and 47.1. In short, the

dominance of the host stem and the gradient of the donor stem are both factors in determining effectiveness of the implant as inductor. In these experiments the implants were $1/4$ sectors of transverse pieces as short as could be cut and were implanted without any attempt at orientation. When isolated, such pieces usually develop into partial apical forms but

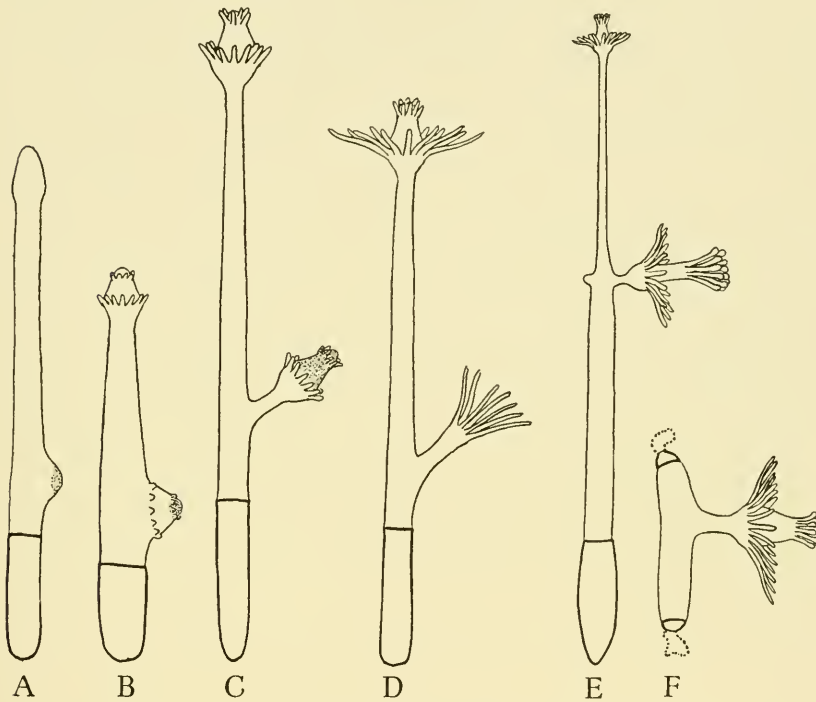


FIG. 127, A-F.—Determination by induction of new axiate patterns by small grafts in *Corymorpha*. A-C, developmental stages, part developing from graft shaded; D, new induced axis consisting of proximal tentacles and stem; E, hydranth-stem axis and outgrowth on opposite side of host stem induced by graft of distal half of fully developed manubrium, reconstituted distal host hydranth inhibited and host stem reduced distal to graft; F, determination of bases at both ends of a piece of host stem by dominance of a new axis induced by a small graft (A-C from Child, 1932c; F, after Child, 1935).

sometimes into complete individuals of extremely small size. When grafted, they usually form only more or less of the apical part of the manubrium. Rarely, however, development of the hydranth is so far inhibited that only proximal tentacles without manubrium develop (Fig. 127, D); in these cases the graft takes part in the tentacle development. With more extreme inhibition only a stemlike outgrowth without hy-

dranth may develop; and at proximal host-levels 10–25 per cent of grafts from proximal donor-levels induce development of basal ends but can also induce hydranth-stem axes. Since the grafts are not oriented when implanted, but the new axis develops approximately at right angles to the host axis, it appears highly probable that the original polarity of the graft plays no essential part in determination. Apparently the graft and perhaps host tissue about it act primarily as a group of active cells and, as such, determine a new dominance and gradient extending into the host tissues. Whether a complete hydranth with stem, proximal tentacles, and stem, stem only, or basal end develops apparently depends on degree of activation of the cells of the graft and surrounding tissue, as determined by level of origin of graft, level of implantation, and inhibiting effect of host dominance. Occasional cases of duplication of hydranth occur in which the graft forms the apical part of one hydranth, the other being formed entirely from host tissue. One case among several hundred has been observed in which the graft lay between two new hydranths and took no part in the formation of either. These duplications are almost completely limited to distal stem-levels, where new axes are determined by simple partial section without implant more frequently than elsewhere and are apparently cases in which sufficient activation of host tissues occurred to determine one, or even two, new axes. In the latter single case, the graft, though not inducing, apparently served as an obstacle to closure of the wound and so brought about more activation and growth of host tissues.

Fully developed hydranths and apical parts of hydranths, when isolated, do not reconstitute proximal parts; but even the distal half of a fully developed manubrium, implanted laterally, can induce the proximal parts of a hydranth and a stem from host tissues (Fig. 127, *E*). Implanted transverse pieces from the middle of fully developed manubria develop hypostome from the distal end, or sometimes from both ends; and both unipolar and bipolar hypostomes may induce a single proximal hydranth region and stem (see also Beadle and Booth, 1938).

In many animals with a new axis developing laterally from graft or incision, the reconstituting host hydranth is more or less inhibited, and the host stem distal to the new axis becomes slender, as in Figure 127, *E*. After it has developed, the new axis can completely inhibit development of a host hydranth at more distal levels within a certain distance and can even induce development of a base from both distal and proximal ends of a piece of host stem isolated with it (Fig. 127, *F*); or, if it is less effec-

tive, it inhibits hydranth development to a greater or less degree at the distal end of the piece (Child, 1935). Development of a base at the distal end of the piece involves complete reversal of polarity; in those forms the dye-reduction gradient is also reversed in direction. Development of a base at the proximal end of the piece also gives evidence of the dominance of the graft hydranth, for it occurs in much higher frequency than in control pieces without graft hydranth. A similar dominance of the hydra bud has been repeatedly observed. If the parent body is cut off a short distance distal to a bud or if a bud forms soon after section, reconstitution is often inhibited, and the bud may become the apical region of the individual (Weimer, 1938; Rulon and Child, 1937).

Reversal of polarity in short pieces of hydroids, planarians, and annelids grafted in inverse position on longer pieces¹⁵ has usually been regarded as resulting from an action of the longer on the shorter component. In view of the evidence for independence of the dominant region it appears improbable that any such action of the longer component is involved in development of an apical region or head on the basal or posterior end of the short piece grafted to the longer. The development probably occurs at the free end simply because the grafted end is not dominant. The case does not appear to be essentially different from development of hydranth or head on the proximal or posterior end of a completely isolated piece that becomes bipolar. On the other hand, development of a basal or posterior part from the distal or anterior end of a short piece grafted to a longer in inverse position is undoubtedly determined by the dominance of the longer component.

The work of Moretti (1911), Gebhardt (1926), and Goetsch (1929) has given evidence of determination of new axes in planarians by grafts, and more recent experiment has demonstrated conclusively the dominance and inductive capacity of both homoplastic and heteroplastic grafts from the region of the cephalic ganglia and from certain other regions.¹⁶ Here only a few of the varied results can be presented. Postcephalic parts do not develop from isolated heads, even when entire; and small pieces from the ganglionic region with cut surfaces on all sides, such as have been used

¹⁵ E.g., Peebles, 1900; King, 1901; L. V. Morgan, 1906; Ruttloff, 1908; Leypoldt, 1910; Korschelt, 1927-31, 1929.

¹⁶ Santos, 1929, 1931, homoplastic, *Dugesia* (= *Euplanaria*) *dorotocephala* and *D. tigrina*, heteroplastic between these species; Okada and Sugino, 1934, 1937, and Sugino, 1938, homoplastic, *Planaria gonocephala*, a Japanese species known by that name; J. A. Miller, 1938, heteroplastic, *D. dorotocephala*, *D. tigrina*, and *D. tigrina novangliae*.

for grafts, die when isolated; but when they are implanted in a planarian body, other parts of the head may regenerate and induction of postcephalic regions in the host body may follow. With complete union of dorsal and ventral epithelia of graft and host on all sides the graft may induce a cylindrical, tubular outgrowth with eyespots variously situated about the closed tip and with either dorsal or ventral epithelium inside or outside; if the outgrowth is dorsal, ventral epithelium is inside; if ventral, it is outside. Under certain conditions, when part of the cut surfaces of graft and host fail to unite, a tubular or funnel-like outgrowth, open at both ends, develops, eyespots may appear in it, and it may induce postcephalic parts in the host. Also, when union between graft and host is incomplete, a normal head may develop from the graft and act as inducer. This is a common result with implantation at postcephalic levels.

Heteroplastic grafts of ganglionic pieces into the ganglionic region give certain results of interest. Complete union with host usually results with ganglionic grafts of *D. dorotocephala* into the ganglionic region of *D. tigrina*. With grafts in normal orientation there is no outgrowth, little development of new tissue, no evidence of induction, and locomotion and reaction to food are normal within 12 hours after operation, though most or all of the host ganglionic region was removed. In controls, with a window in the ganglionic region similar to that in which the graft is implanted, normal reactions appear only after a week or more of reconstitution. Apparently, the implanted ganglionic region of the one species attains dominance very rapidly in the other. With reversed orientation of the heteroplastic grafts complete union is less frequent, tubular outgrowths with eyespots may develop, some with short postcephalic induction, and a secondary head may develop from a free cut surface of the host. These forms often show further modifications resulting from partial distintegration or resorption of host head or graft outgrowth and may finally approach more or less closely a normal animal with single head. Apparently either the graft head or the host head may dominate the other component and inhibit it. Even with dorsiventral reversal of heteroplastic grafts in the ganglionic region complete union may result. Such animals advance with head raised and bent posteriorly. With incomplete union a graft head or combinations of graft and host tissue may develop. Extra eyespots frequently appear with ganglionic grafts in the ganglionic region, even though there is no outgrowth, and with other than normal orientation of the graft its polarity may be altered or reversed. These results with ganglionic grafts in the ganglionic region are from Miller.

Homoplastic and heteroplastic grafts of ganglionic pieces into the anterior prepharyngeal region (Santos, Okada, and Sugino) may induce short postcephalic regions, the longest about equal in length to the distance between level of implantation and host head (Fig. 128, A-C). Grafts at the pharyngeal level usually induce a prepharyngeal outgrowth and a pharynx in the host body posterior to the outgrowth (Fig. 128,

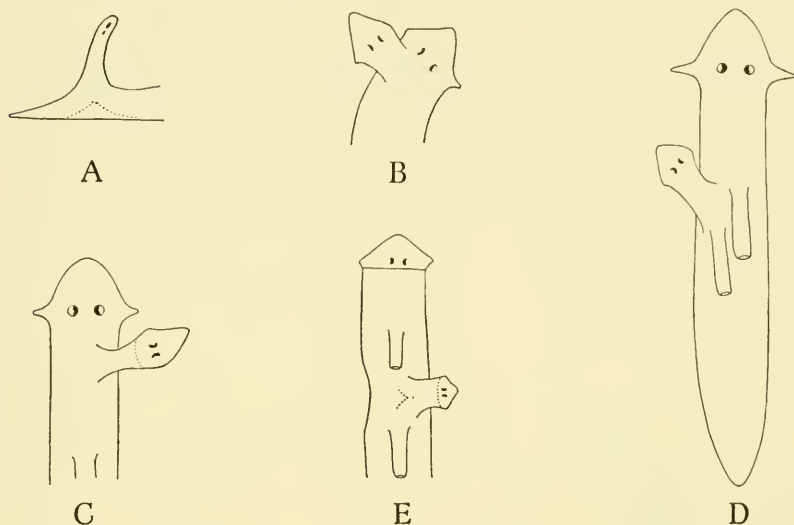


FIG. 128, A-E.—Ganglionic grafts of *Dugesia* (= *Euplanaria*): regeneration of graft and induced reorganization of host. A, homoplastic, *D. dorocephala*, in anterior prepharyngeal region, has induced cylindrical outgrowth with dorsal epithelium on outer surface; B, heteroplastic, *D. tigrina novangliae* to *D. dorocephala*, host decapitated twice; C, heteroplastic, *D. tigrina novangliae* to pharyngeal region of *D. dorocephala* with removal of host pharynx with old pharynx remaining, induces prepharyngeal region and pharynx; E, heteroplastic, *D. tigrina novangliae* to pharyngeal region of *D. dorocephala* with removal of host pharynx and head, induces prepharyngeal region and pharynx posterior to graft, and regenerating host head induces another pharynx anterior to the graft (from Santos, 1929, 1931).

D, E). Other examples of induction by heteroplastic grafts in the pharyngeal region, showing the induced modifications of the alimentary tract, appear in Figure 129 from Miller's experiments. Details are given in the legends. In A, B, and C of Figure 129 a pharynx is induced posterior to the graft, and in B and C regeneration of a host head after later decapitation anterior to the graft-level is completely inhibited; the two branches of the alimentary tract at the original anterior end after this decapitation in B indicate its reorganization into a posterior end. In Figure 129, D, the graft has induced a pharynx posteriorly, another anteriorly with re-

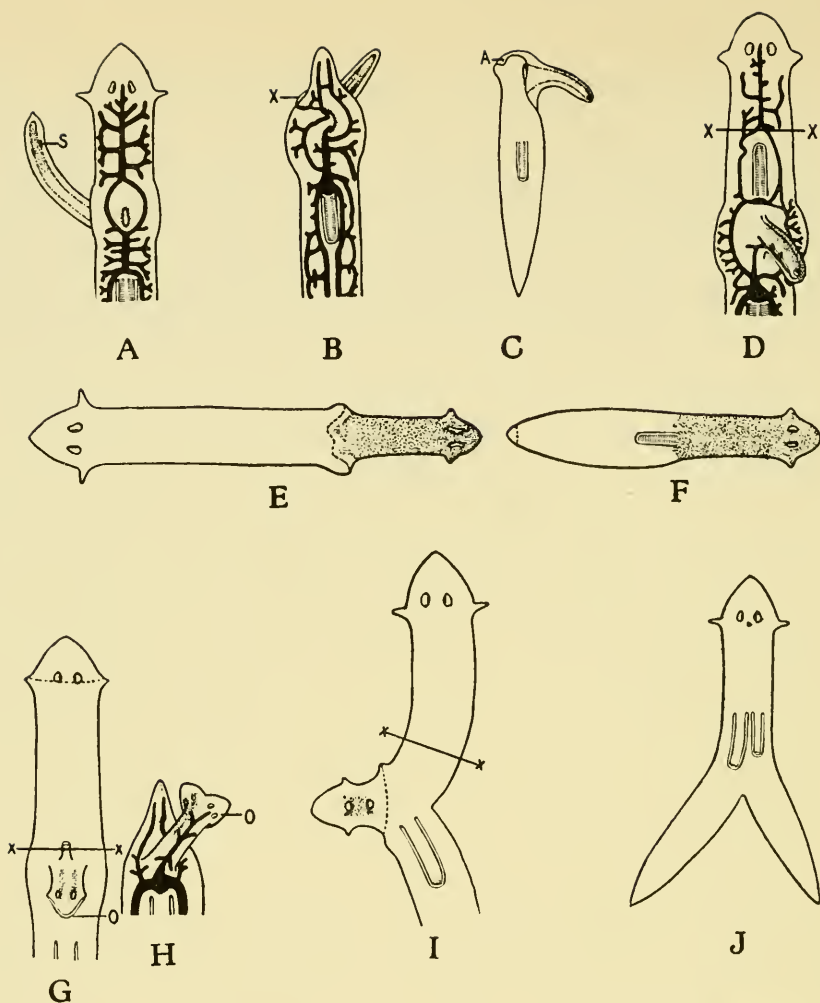


FIG. 129, A-J.—Heteroplastic grafts in pharyngeal region. A, E, F, *Dugesia tigrina* to *D. dorotocephala*; B, G-J, *D. tigrina novangliae* to *D. dorotocephala*; C, D, *D. dorotocephala* to *D. tigrina novangliae*. A, DDAA* union complete, 42 days; tubular outgrowth developed ventrally, covered with ventral epithelium and with two supernumerary eyespots, one shown at S. B, DDAP, union complete, 35 days; original eyespots disappeared and four pigmented areas developed, tail-like outgrowth from right side of host anterior to graft; host, decapitated at X, failed to regenerate. C, DDAP, union complete, 42 days, ventral view; tubular outgrowth with terminal eyespots from large graft; host, decapitated about 3 mm. from graft, regenerated only small amount of new tissue at A. D, DDAP, large graft union, complete, 28 days; pharynx with reversed polarity induced anterior to graft; extent of graft dominance anteriorly indicated by anterior reorganization of digestive tract; after section at XX host failed to regenerate. E, DDAA, large graft, 31 days, union complete, but graft given a free posterior surface by section 7 days after implantation; with development of head from posterior side of graft its polarity is completely reversed. F, the animal of E at 74 days; the graft has retained specific characters and has reversed polarity in a part of the host, as indicated by ciliary movement, orientation of pharynx, and regeneration of a tail after fission from a level originally anterior to the graft. G, DDAP, 14 days; anterior surface of graft failed to unite with host; head (O) has developed from host portion of tubular outgrowth and small pharynx has been induced anterior to the graft. H, the posterior part of the animal shown in G after section at XX, with posterior end regenerated from the originally anterior surface of section. I, DDAA, 21 days; left side of graft failed to unite with host and host lateral to graft tore apart; modification of graft polarity indicated by position of auricles in relation to the eyespots. J, following decapitation of I at XX, tail and second pharynx were induced in host anterior to the graft (from J. A. Miller, 1938).

* In this figure and in Fig. 131 orientation of the graft in relation to the host is given in the notation adopted for amphibian limb grafts by Harrison (p. 390); "DDAA" means that dorsal and anterior sides of graft are toward dorsal and anterior of host, that is, the graft is normally oriented; "DDAP," graft in normal dorsiventral, reversed anteroposterior orientation.

versal of host polarity and reorganization of digestive tract. After removal of the anterior region of the host at XX, following development of the reversed pharynx, head regeneration was inhibited. In Figure 129, *E* and *F*, is shown a case of complete reversal of host polarity and also reversal in the graft, because it was given a posterior free surface after union, by removal of regions posterior to it. In *G* and *H* a pharynx is induced both posterior and anterior to the graft, and later removal of the host anterior end is followed by development of a posterior end instead of a head. In *I* polarity of the graft is altered 90° because its free surface, from which the head develops, was originally lateral, a pharynx is induced posteriorly, and later section of the host at XX is followed by induction of another pharynx at a host-level originally anterior to the graft-level and development of a posterior end from the originally anterior cut surface (*J*).

Ganglionic grafts in the posterior zooid region very generally induce a postcephalic outgrowth and reorganization of the host body posterior and for a certain distance anterior to the graft. Two examples are shown in Figure 130, and others with heteroplastic grafts and showing reorganization of the digestive tract in Figure 131. *A* and *B* show induction in both directions, though the graft head is far from normal in both; *C* is the posterior product of fission following induction by the graft both posteriorly and anteriorly, with development of posterior end instead of head from the originally anterior surface of fission (*A*). In this individual gradual fusion of the two posterior ends resulted in the condition of Figure 131, *D*, with two pharynges at the same body-level and with a duplicated postpharyngeal digestive tract. Later fissions in cases

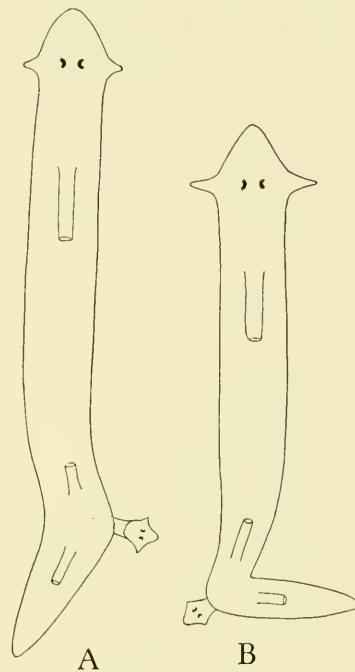


FIG. 130. *A, B*.—Induction by ganglionic grafts in posterior zooid region. *A*, homoplastic, *Dugesia tigrina novangliae*; induced postcephalic tubular outgrowth and reorganization of host body with development of pharynx posterior and anterior to the graft-level. *B*, heteroplastic, *D. tigrina novangliae* to *D. dorotocephala*; induction by graft of postcephalic outgrowth and of reorganization of host with pharynx development posterior and anterior to graft-level (from Santos, 1929, 1931).

of this kind may show duplication of eyespots and of prepharyngeal, as well as of postpharyngeal, regions. One case observed showed duplication through sixteen successive fissions (Miller).

A point of considerable interest is that the induced reversal of polarity in the host is a gradual progressive process, extending over several weeks.

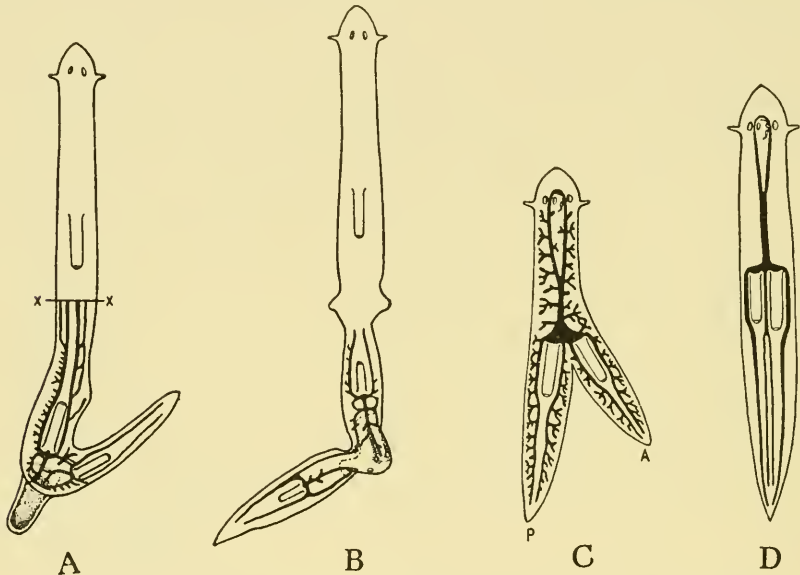


FIG. 131, A-D.—Heteroplastic planarian ganglionic grafts in posterior zoid region. A, B, *Dugesia tigrina novangliae* to *D. dorotocephala*; C, D, *D. tigrina* to *D. dorotocephala*. A, DDAP, union complete, 21 days; outgrowth, reorganization of digestive tract and development of pharynx posterior and anterior to graft; after section at XX tail instead of head developed on piece containing graft. B, DDAP, union complete, 14 days; induction of reorganization and pharynx development posterior and anterior to graft. C, DDAA, 133 days; induced reorganization and pharynx development posterior and anterior to graft. After fission at A, originally anterior to the graft, a tail instead of a head reconstituted, and this part became a second posterior end, as in the figure, and fusion between the two posterior ends A and P gradually took place. D, same animal as C at 216 days, after fusion of the two posterior ends; only main branches of digestive tract are shown (from J. A. Miller, 1938).

Section anterior to the graft a few days after it is implanted is usually followed by regeneration of a normal head. Section at a later period may result in a differentially inhibited head or complete inhibition of regeneration, and after still later section a posterior end instead of a head develops. Evidently there is a gradual extension anteriorly of the graft dominance until it is blocked by the original dominance of the host. In the light of other data this gradual extension of dominance suggests a

gradual reorganization of the nervous system, progressing from, and induced by, the dominant region of the graft. Doubtless a similar, though less extreme, reorganization is induced posterior to the graft; but since it is in the direction of the original polarity, its progress is not so readily shown. According to Miller, the epithelium and pigment of heteroplastic grafts are, in most cases, gradually replaced by epithelium and pigment characteristic of the host. To what extent other tissues are replaced is not known.

The extensive experiments of Okada and Sugino with a Japanese planarian species show essentially the same relations of dominance and induction by the grafts, but they have also shown that the head region anterior to the eyespots and pieces from the lateral head region, including an auricle, are capable of dominance and induction and that postcephalic pieces from the prepharyngeal region can induce a pharynx even without head regeneration. Both the anterior and lateral pieces of the head include some of the central nervous system, and the inducing capacity of prepharyngeal pieces is what might be expected from the fact that isolated prepharyngeal pieces that remain completely acephalic reconstitute all parts posterior to their level of origin in the parent body (p. 180) but nothing anterior to that level.

Working with European planarians, Brøndsted (1939) finds that entire, fully developed heads grafted on anterior cut surfaces of planarian pieces do not induce reorganization. He maintains that the new tissue of regeneration, the blastema, is the inducing agent. With the American species, however, very little new tissue is formed in many cases in which the grafts induce, and in the experiments of Santos and Miller no evident relation between amount of new tissue and induction has been found. It is entirely possible, however, that fully developed heads of some planarian species are not active enough to induce reorganization.

MIRROR-IMAGING IN VARIOUS RECONSTITUTIONS

The two opposed components of bipolar forms of *Tubularia*, *Corymorpha*, planarians, and annelids are, in general, mirror images of each other because, although their polarities are opposed, the radial, ventrodorsal, or bilateral patterns are essentially the same. Under certain conditions reconstitution may result in triplication of axes. The actinian *Harenactis* affords an excellent example. A partial transverse section involving both body wall on one side and one side of the esophagus results in union of the cut surfaces of body wall and esophagus both distal and proximal to

the level of section, leaving a lateral opening into the esophagus. As Figures 132, *A* and *B*, show, there is a complete disk and tentacles at the distal end of the body (*D*), either the original or a reconstituted oral end. Distal to the lateral opening a partial disk and partial circle of tentacles are reconstituted with a polarity opposed to the original (*D'*), and proximal to the opening another partial disk and tentacles with polarity in the original direction but, of course, with an alteration of the original polarity (*D''*). Distal to the opening, one side of the animal is bipolar, and the partial oral end *D'* is a mirror image of *D''* and also of the cor-

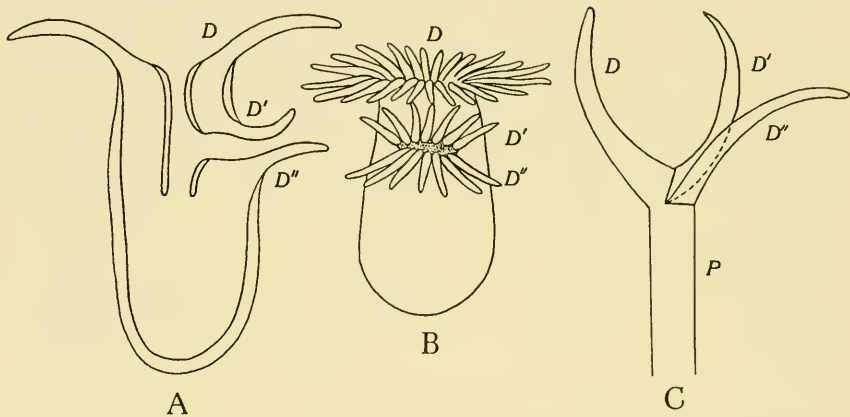


FIG. 132, *A-C*.—Mirror-imaging in reconstitution, *A*, *B*, in *Harenactis attenuata* (from Child, 1909*b*); *C*, schematic outline for *Bruchdreifachbildungen*; explanation in text.

responding part of *D* (Child, 1909*b*). Similar reconstitutions can, of course, be produced in other forms.

A large number of triplications with similar mirror-imaging, mostly appendages, have been described by Bateson (1894, chap. xx) and Przibram (1921 and earlier papers). Some of these have been produced experimentally by lateral partial section; others have been found as anomalies. These triplications have been called *Bruchdreifachbildungen* by Przibram. He regards them as resulting from a lateral injury with reconstitution of distal parts from both distal and proximal surfaces of the injured region, essentially like the case of *Harenactis*. Figure 132, *C*, indicates diagrammatically the character of these triplications, the curvatures of the triplicate distal parts representing the asymmetries. The mirror-imaging is the same in *D*, *D'*, and *D''* in Figure 132, *A*, *B*, and *C*, the only difference between the *Harenactis* triplication and that of the appendage

being that in the former D' and D'' are partial symmetries developing in relation to the anatomy of the original individual, while in the latter the asymmetries of the reconstituted parts are complete, even though they develop from surfaces of partial section.

Przibram interprets these triplicate forms in terms of a hypothetical space lattice (*Raumgitter*) in the protoplasm. In terms of the gradient concept, activation of the two surfaces by section or injury is sufficient to determine a new polar gradient on each. With outgrowth of new tissue each becomes the distal part of an appendage, the parts regenerating being those normally distal to the level of the appendage from which regeneration takes place, though in some cases not all of these develop. Evidently the polarity of D' originates independently of the polarity of the region from which it develops; and if the preceding analysis of reconstitution is not wholly mistaken, the polarity of D'' is also independent of that of the region from which it develops. The asymmetries of the regenerates, D' and D'' , however, are the same as the asymmetry of the region from which they develop, except that their asymmetry may be complete and normal, although they develop from a surface of partial asymmetry. Apparently the partial asymmetry differential at the surface from which regeneration takes place is sufficient to determine a complete asymmetry in the new tissue.

The regenerated axes, D' and D'' , do not usually develop parts proximal to the level from which they arise. In the case of D'' these parts are already present; as regards D' , if the high end of the gradient is distal and development progresses proximally, it is balanced and its further progress prevented by the similar opposed axis D ; if development progresses distally from the surface of section or injury, development of distal parts is probably determined by the activation adjoining that level; if the high ectodermal region is distal and that of the mesoderm proximal, both these factors may be concerned in determining the result. The scale of organization of D' is often smaller than that of D , probably in consequence of more or less inhibition of the new gradient by D . A similar difference in scale appears very often in bipolar partial forms of hydroids and planarians, the proximal hydranth or partial hydranth and the posterior head being smaller and less fully developed and sometimes representing a smaller part of the polar axis than the distal hydranth or the anterior head.

ASYMMETRY AND MIRROR-IMAGING IN RECONSTITUTION
OF AMPHIBIAN LIMBS

A new limb polarity, opposite in direction to the original, may originate in regeneration of amphibian limbs. A piece of limb including the knee and part of the upper and lower leg implanted in a host body by its distal cut end may show regeneration from its free proximal end, but this regenerate gives rise not to parts normally proximal to the level of the proximal cut end on which it appears but to the distal portion of a limb, which may include all levels normally distal to the proximal cut end from which it arises. Its polarity is opposite in direction to that of the original piece and evidently independent of it, but the asymmetry is the same as that of the proximal stump. Evidently the asymmetry provides a pattern at the cut end, and this affects or persists in the regenerating tissue; but there is no such pattern for the polarity of the regenerated part, and this evidently represents a new pattern originating in the bud of new tissue. The regenerated part is a mirror image of the reversed piece except that it develops distal parts absent from the piece.¹⁷ Similarly, pieces of amphibian tails implanted in the dorsal region by their posterior cut ends may become bipolar by regeneration of a new posterior end from the free anterior cut end (Milojević et Burián, 1926). Here, also, the symmetry of the regenerated posterior end is the same as that of the implanted piece, but the polarities are opposed; consequently, there is mirror-imaging, so far as parts are present in the implanted piece.

Development of limbs following transplantation of limb primordia in amphibia presents a number of interesting problems and an indefinite range of experimental possibilities; a great variety of experiment and an extensive literature have resulted.¹⁸ According to the terminology adopted by Harrison (1921a), transplantation is orthotopic to the normal location of the limb, on either side of the body, or heterotopic to some other location—for example, the flank, homopleural to the same side of the body as origin, heteropleural to the opposite side. As regards orientation of the transplanted primordium, dorsodorsal position is with dorsal border dorsal, dorsoventral, with dorsal border ventral with respect to the host. The anteroposterior axis of the transplant coincides in direction with that

¹⁷ Della Valle, 1911, 1913; Kurz, 1922a; Gräper, 1922; Milojević et Grbić, 1925.

¹⁸ Harrison, 1921a, 1925a, and Przibram, 1924, 1927, give numerous references; the general review by Mangold, 1929a, gives a very complete bibliography. See also Swett, 1923, 1926, 1927, 1928a, b, c, 1930, 1932, 1937a, b, 1938a, b, c, 1939; Oka, 1934, and references cited by them. Experimental data concern chiefly the forelimb.

of the host when transplantation is homopleural dorsodorsal or heteropleural dorsoventral and is reversed in homopleural dorsoventral and heteropleural dorsodorsal. These relations give four positions of transplant with respect to host axes in orthotopic location, as indicated in Figure 133, and four similar positions in lateral heterotopic location.

Experimental results with *Amblystoma punctatum* led Harrison to formulate three rules, of which the essential points are as follows: (1) reversal in relation to the host of the anteroposterior axis of the limb bud results in a limb with asymmetry of the side opposite that on which the bud was implanted (disharmonic); (2) when the anteroposterior axis is not reversed, the asymmetry of the resulting limb is that proper to the

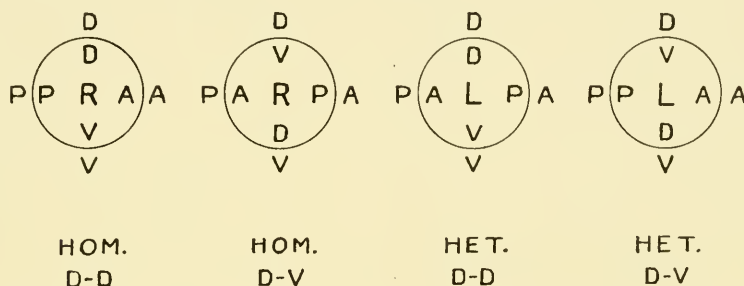


FIG. 133.—Diagram of positions of amphibian limb-bud transplants in relation to host axes. A, anterior; P, posterior; D, dorsal; V, ventral, of host outside, of limb bud inside circle; R, right; L, left; Hom. D-D, homopleural dorsodorsal position, etc. (from Harrison, 1921a).

side on which it is located (harmonic); (3) the original member (the first to begin development) of limb duplications has an asymmetry according to (1) or (2), and the secondary member is a mirror image. In later experiment these rules have been found to hold very generally, few real or apparent exceptions having been observed.

Duplication, with mirror-imaging of the two limbs, is frequent, and in some cases there is triplication, in which case each limb is usually a mirror image of the one next it; that is, there are two planes of mirror-imaging, as indicated in Figure 134, but certain cases have been observed in which this relation apparently does not hold (Swett, 1924; Oka, 1934). In orthotopic location duplications are more frequent when axial relations between bud and host are disharmonic; in heterotopic locations they are more frequent when relations are harmonic; but little is known concerning the physiological factors that bring about duplications and triplications.

As regards the asymmetry relations, Harrison holds that anteropos-

teriority is definitively established in the primordium or bud at the time of transplantation but that dorsiventrality is not yet established or is reversible until later stages, the dorsiventrality of the transplant being de-

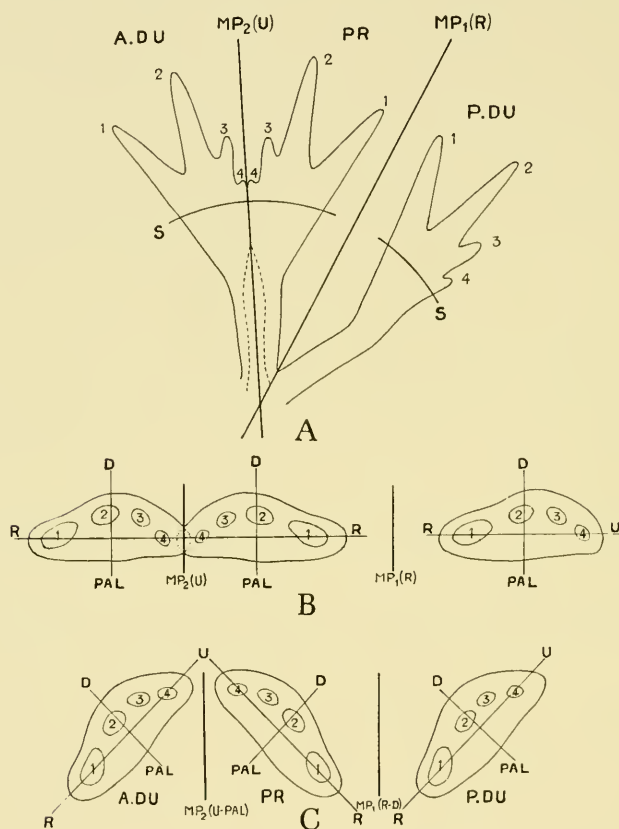


FIG. 134, A-C.—Diagrams of limb reduplication. A, PR, primary limb; P. DU, posterior reduplicating member; A. DU, anterior reduplicating member; $MP_1(R)$, primary (radial) mirror plane; $MP_2(U)$, secondary (ulnar) mirror plane; 1-4, digits; S, level of section shown in B and C. B, C, sectional diagrams of reduplication; in B mirror planes radial (MP_1) and ulnar (MP_2); in C they are radiodorsal (MP_1) and ulnopalmar (MP_2). D, dorsal; PAL, palmar; R, radial; U, ulnar (from Harrison, 1921a).

terminated by the dorsiventrality of the host. When double or triple limbs develop, the mirror-imaging results, according to Harrison, from development of more than one growth center within the range of mutual influence. The center developing first, or most advantageously placed, reverses the asymmetry of the other or others. Localization of growth cen-

ters beyond the range of mutual influence may result in duplication without mirror-imaging.

A very different interpretation has been advanced by Przibram (1924, 1925, 1927). His view is that axiate pattern is definitively established in the primordium at the time of transplantation, that apparent reversal of asymmetry is not due to an influence of the host body or pattern but to development from the proximal, instead of the distal, part of the transplant. With persistence of the original asymmetry such a limb will be a mirror image of a limb developing from the distal region of the limb bud, just as a proximal hydranth or a posterior head or an isolated hydroid or planarian piece is a mirror image of that at the distal or anterior end. Such a limb would correspond to D' of Figure 132, *C*. Duplication would result from development of a limb both distally and proximally from the transplant, that is, from bipolar development with original asymmetry in both polar axes. Triplication of limbs Przibram interprets in terms of the *Bruchdreifachbildungen* (p. 388), as limb development from distal and proximal regions of the transplant and also from the limb bud region of the host. It may be noted that Della Valle (1911, 1913) obtained the axial relations of Figure 132, *C*, in regeneration from a partial transverse section of the amphibian limb.

As in so many biological controversies concerning interpretations, it seems entirely possible that both views may be correct, that is, that duplications and triplications of limbs and their asymmetries may occur in both ways and perhaps also a new center of limb development, that is, a region of activation may sometimes originate in a region of less complete union of transplant and host or of more intense irritation by the operation. There are plenty of cases in which such factors do initiate axiate development. It is well known that parts of limb primordia sectioned in various directions may give rise to whole limbs.¹⁹ Limb-bud mesoderm isolated from ectoderm and implanted with reversal of the mediolateral axis, the longitudinal axis of the limb, results in development of whole limbs which accord with the rules (Harrison, 1925*a*). Reduplicated limbs developing in heteroplastic transplants between *Amblystoma* species always have donor characteristics, according to Swett (1932). On the other hand, Oka (1934) maintains that in *Hynobius* some cases of duplication represent distal and proximal regeneration from the transplant and that some triplications develop entirely from the transplant.

¹⁹ Harrison, 1921*a*; Swett, 1924, 1926, 1928; Gräper, 1926.

Harrison regards the asymmetry of the limb as an expression of an "intimate structure" and suggests an analogy to compounds composed of asymmetric molecules. Przibram (1921) postulates a space lattice which is assumed to reverse its asymmetry as required by experimental data. At present, however, there is no evidence of the existence of such an oriented intimate structure or space lattice as the basis of axiate pattern; and the mechanism of reversal of such a structure in a limb-bud transplant by some effect of the host pattern, or in relation to another bud, requires further hypothesis. Interpretation in dynamic terms seems to offer less difficulty. There is an anteroposterior and a dorsiventral differential in susceptibility and in rate of dye reduction in the amphibian embryo at stages corresponding to those used in limb transplantation. Pigmentation has made it impossible with the material at hand to demonstrate a gradient in rate of dye reduction in the limb bud, but in the chick limb bud such a gradient is clearly evident (Rulon, 1935). The limb primordium must share in the gradient pattern of the body, but the dorsiventral differential in it, though apparently present in gastrula stages (Swett, 1938c, 1939), can be altered by change in orientation with respect to the host pattern up to much later stages. The dorsiventrality of a small transplant is alterable up to a later stage than that of a large transplant (Lovell, 1937). Larger limb-disk transplants of *Triturus* are more likely to develop than smaller. With transplantation to the mid-ventral line duplications are usually ulnar and in a plane transverse to, but on the flank are usually radial and in the plane of, the longitudinal body axis. Inclusion in the transplant of parts about the limb primordium, particularly the region dorsal to it, results in better limb development (Takaya, 1938). Here, as in *Hydra* and other invertebrates, size of transplant appears to be a factor in its persistence, and the body pattern is apparently a factor in determining not only plane of duplication but direction of radio-ulnar asymmetry.

The frequent rotations of limb-bud transplants in other than normal orientation noted by various authors (e.g., Nicholas, 1924) suggest presence of effective dynamic factors. If such factors can determine rotation in many cases, it is not difficult to conceive that they may determine a physiological axiate pattern in a transplant that does not rotate. Moreover, since a dynamic factor of some sort is apparently required to bring about reversal of an intimate structure or space lattice, it is at least a pertinent question whether it is necessary to postulate the structure. As regards duplication and mirror-imaging, the effects of two dynamic fields

on each other are perhaps of interest. These effects have been pointed out by various authors in attempts at analysis of the mitotic figure. For example, if two like magnetic poles are brought near together, the field of each is altered so that the two become mirror images of each other. If, then, a third like pole is brought near on one side, so that it acts equally on both fields, both acquire, so to speak, a dorsiventrality but are still mirror images of each other. Similarly, two equal fields of fluid, each flowing radially from a center, when near together alter each other so that each becomes a mirror image of the other. A current flowing at right angles to the line joining the two centers will determine a new asymmetry in both fields, but they will still be mirror images. In both the magnetic and the fluid fields a morphological asymmetry and mirror-imaging is established and persists as long as the dynamic factors act; or if the magnetic field is made visible by iron particles, the pattern may persist after removal of the poles. These examples of field action and resulting pattern are offered merely as suggestions, not as models.

Apparently reversal of asymmetry in one limb primordium by another may depend on relative positions of the two. With transplantation in certain orientations to a position anterior to the host limb bud, the asymmetry of the transplant is reversed; with transplantation in normal orientation posterior to the host limb bud, asymmetry of the host limb is reversed. In these combinations the posterior member always develops more rapidly and the anterior member is reversed, apparently by the dominance of the other (Takaya, 1936). These experiments suggest that position in the anteroposterior body gradient may influence rate of development and that developmental activity, rather than a structural pattern, is the essential factor in influence of one limb bud on another. In this connection mirror-imaging of sea-urchin larvae developing from blastomeres of the two-cell stage in contact after separation (Driesch, 1906) and the cases of situs inversus and mirror-imaging in twinning and double monsters are of interest, as probably involving much the same factors as amphibian limbs. In hydroid reconstitutions two hydranths developing close together often show more or less inhibition on the facing sides and so are mirror images.

REGENERATION OF THE LENS IN AMPHIBIA

The regeneration of the lens from the dorsal region of the iris in various amphibia has presented a problem of considerable interest which has led

to repeated experiment and various interpretations.²⁰ This regeneration occurs not only after the lens is removed from the eye but after it is displaced so that direct continuity with the iris is lost and after lens degeneration by X-radiation (Poltzer). The fact that the origin of the regenerated lens is different from its embryonic origin and the apparent limitation of potency to the upper or dorsal part of the iris led Wolff to a teleological interpretation which was opposed by Fischel, but many investigators have concerned themselves chiefly or wholly with presentation of data. Sato (1930), transplanting pieces of the upper iris to another eye, found a gradient of decreasing lens potency from the most dorsal iris region. In further experiments he implanted optic primordia of different embryonic stages with dorsiventral reversal, so that the originally dorsal side of the optic cup was ventral; and at a later stage shortly before metamorphosis, he removed the lens (Sato, 1933*a*, *b*). According to the stage of dorsiventral reversal, the choroid fissure developed ventral in position, dorsal in origin, or two fissures developed, or the original dorsiventrality persisted. Apparently, dorsiventrality of the optic primordium is not irreversibly fixed in earlier stages; in later stages the original dorsiventrality apparently determines one fissure, the imposed dorsiventrality another; still later, the fissure develops in its normal position with respect to the original dorsiventrality. The lens regenerates from the iris region opposite the choroid fissure or, when two fissures are present, opposite the more completely developed; and a gradient of lens potency decreasing from the iris region opposite the fissure is present. Sato concludes that the choroid fissure exercises an inhibiting action on lens regeneration, so that the lens arises from the iris region farthest from it.

Sato's data, as well as those of others, suggest a more general interpretation, that is, that both position of choroid fissure and region of lens regeneration are expressions of dorsiventral pattern, evidently a dorsiventral gradient, either the original gradient or that imposed on the implanted, dorsiventrally reversed eye. The high region of the dorsiventral

²⁰ Colucci, 1891; Wolff, 1895, 1901, 1913; E. Müller, 1896; Fischel, 1900*a*, 1902; Spemann, 1905; Wachs, 1914, 1920; Ogawa, 1921; Beckwith, 1927; Adelmann, 1928; Sato, 1933*a*, *b*, 1935; Törö, 1932; Kesselyák, 1936; Poltzer, 1936; Monroy, 1939. Lens regeneration from the iris has been observed in adults of certain urodeles; but in *Amblystoma punctatum* and *A. tigrinum* and in the anurans *Rana clamitans*, *R. pipiens*, and *R. sylvatica* it does not occur even in larval stages; in these forms, however, lens fragments implanted or remaining after lens removal may regenerate lens (Stone and Sapir, 1940, "Experimental studies on the regeneration of the lens," *Jour. Exp. Zool.*, 85).

gradient in vertebrates is dorsal, and the developing eye may be expected to possess a dorsiventral differential representing a part of this gradient. According to this suggestion, lens regeneration occurs normally from the dorsal iris margin, and lens potency decreases ventrally from this region, because the dorsal region represents the highest gradient-level present in the eye and reacts most rapidly to removal of the lens, and probably also exercises some degree of dominance over lower levels. In earlier stages the dorsiventral differential is reversible, but perhaps becomes specific later, though it is possible that even in the adult the difference is one of susceptibility or reactivity. Tests of sectors of the iris of reversed eyes for lens potency show that the potency of the dorsal region (originally ventral) is not very high and not widely different from that of other parts. This suggests that the original dorsiventral gradient and that imposed secondarily by reversal partially neutralize or obliterate each other, and Sato's experiments in general indicate that either may become the effective one. That a dorsiventral differential is involved in lens regeneration is further suggested by the relation of orientation of the lens fibers to the dorsiventrality of the optic cup (Dragomirow, 1930).

Interpreted in these terms, lens regeneration does not appear to differ fundamentally from the decreasing capacity for head regeneration from higher to lower levels of the polar gradient in various planarians and annelids and the disappearance of the capacity at certain levels in some forms. In all these cases it still remains to be determined whether the apparent decrease and disappearance of potency at lower gradient-levels results from specific differentiation or from a difference in susceptibility or intensity of reaction to the experimental conditions. The reversal of dorsiventrality of the eye implanted in dorsiventrally reversed position is paralleled by the reversal of dorsiventrality in similarly reversed, transplanted limb buds.

DOMINANCE AS AN INHIBITING FACTOR

In both plants and animals a dominant region tends to inhibit development of another similar region within a certain distance of itself. Various instances of this effect of dominance have already been discussed in other connections. Here a few further cases are considered in which dominance either prevents a certain kind of reconstitution, inhibits it to a greater or less extent, or brings about regression or destruction of parts already morphologically distinguishable.

INDUCED REGRESSION IN CERTAIN HYDROIDS

Removal of the distal half of the hydranth primordium of *Tubularia* (see Fig. 13 [p. 36]) in its earlier stages may lead to complete regression of the proximal part and development of a new complete primordium in the usual relation to the end of the piece. In this case the gradient is altered by the activation following the second section, and a new hydranth pattern is determined, partly in the cells forming the proximal part of the first primordium, partly in cells farther proximal. The new pattern obliterates that which had already begun to develop. Results of such operations, however, differ with level of section and stage of development of the first primordium. If section is near the distal end, only more or less reorganization of the distal region of the primordium may occur; or if the primordium is advanced in development, regeneration of the part removed is the usual result. Regression of the primordium sometimes results in both *Tubularia* and *Corymorpha* from section immediately proximal to it in early stages or in its proximal region. Disappearance of the original primordium is followed by development of a shorter one in the distal region of the piece and, in some cases, of another at the proximal end. In these cases activation following section at the proximal end of the early hydranth tends to establish a new dominance and reverse the gradient pattern over more or less of its length; and the reversal obliterates the former pattern, even though it has become morphologically visible, and determines a new pattern at one or both ends.²¹

REGRESSION OF FISSION ZONES

Fission may be inhibited and the fission zone obliterated in planarians by section and regeneration of a head a short distance anterior to the zone. Here there is no directly visible regression, but the region which represented the anterior part of a posterior zooid is reorganized into a prepharyngeal region. If or when fission occurs in such an individual after reconstitution, it is at a level much farther posterior. In some species of *Stenostomum* section and reconstitution of a head a short distance anterior to a fission zone already visible, but in early developmental stage, will bring about regression of the zone and reorganization of the region concerned into a part of the body of the reconstituted individual. Similar regression and reorganization of early fission zones has been brought about in the same way in several species of microdrilous oligochetes by various experimenters. A section anterior to the zone is much more effective than posterior section (E. H. Harper, 1904).

²¹ Driesch, 1897, 1902b; Peebles, 1900; Child, 1909d.

INHIBITION OF RECONSTITUTION AND DESTRUCTION OF ZOOIDS
AS A RESULT OF ALTERED DOMINANCE

According to Stevens (1902), pieces from the basal stem region of *Antennularia ramosa* usually reconstitute hydranth-stem axes; those from more distal levels, stolons. This difference appears to be due to different relations of dominance at the different levels. At the proximal levels hydranths are often absent from the lateral branches or in poor condition, while farther distally they are present and in better condition, the more distal branches being physiologically younger and their dominance more effective in inhibiting development of hydranth-stem axes than that at more proximal levels.

In planarian reconstitution a developing head region may, under certain conditions, inhibit development of another head. For example, in the well-known experiment involving decapitation and partial anterior longitudinal splitting of the body, a head develops on the anterior end of each separated part, even if the two anterior ends represent different body-levels (Fig. 135, *A*, *B*). However, if a half-transverse section is also made at the posterior end of the longitudinal split, thus removing one of the two separated parts, head development is inhibited on the half anterior cut end resulting from this section (Fig. 135, *C*). Apparently, in this case the more posterior level of section on the one side is dominated and determined as lateral, instead of as head, by the dominant region at a more anterior level on the other side. In the case of *B* this dominance is not effective in inhibiting head development, probably because it cannot overbalance the gradient already established and intensified by the activation following section in the shorter anterior end. This experiment has been used repeatedly by the writer in laboratory class work (see also Goldsmith, 1939). The conditions in forms like Figure 135, *A* and *B*, are comparable to those in bipolar forms of hydroids and planarians; neither dominant region affects the other because the two opposed gradients are more or less nearly equal. In the case of Figure 135, *C*, one of the opposing gradients has been removed, and head development is inhibited. It has been shown earlier in this chapter that planarian head reconstitution can also be inhibited by grafts.

A striking case of the effect of altered dominance appears in the inhibition, disintegration into cells, and complete destruction of zooids already morphologically distinguishable in certain species of the rhabdocoel *Stenostomum* (Child, 1903*b*; Van Cleave, 1929). In Figure 136, *A*, a chain of *S. grande* sectioned at *XX* leaves the posterior part of zooid *1.1*. and

the whole of zooid *1.2.* with head in early developmental stage, anterior to zooid *2.1.* with more advanced head. Although the anterior end of the posterior part of zooid *1.1.* is capable, under other conditions, of head development, it is completely inhibited, and a gradual reduction and sepa-

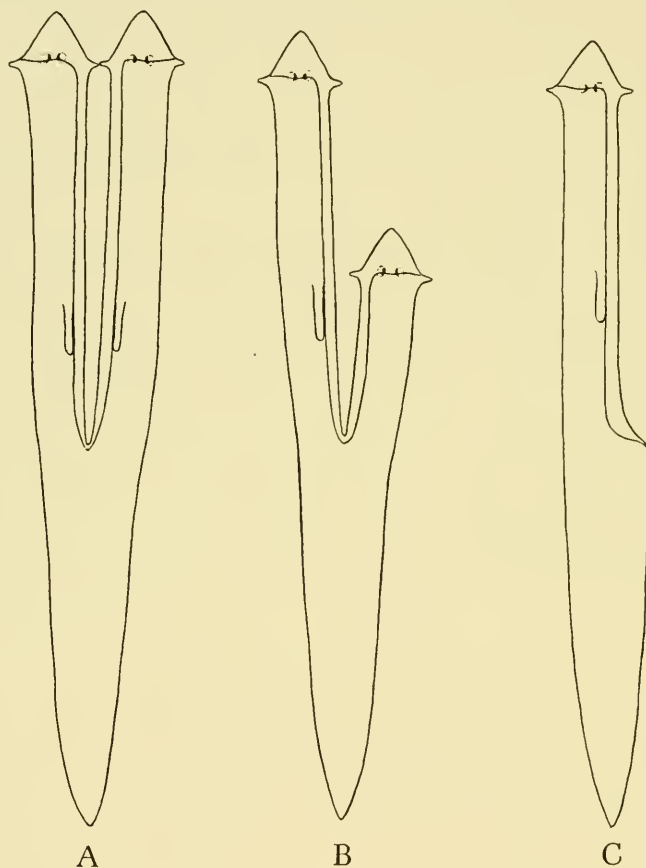


FIG. 135, A-C.—Planarian head regeneration at different levels, following decapitation and anterior partial longitudinal splitting; explanation in text.

ration into cells, first of the part of zooid *1.1.*, then of zooid *1.2.*, results (Fig. 136, B-E). The cells from these zooids accumulate in the pseudocoel and gut of the dominant zooid *2.1.* and in zooid *2.2.* posterior to it and gradually disappear, apparently serving as nutritive material, for these zooids grow rapidly. That the head region alone of an older zooid can bring about this destruction of headless and younger complete zooids

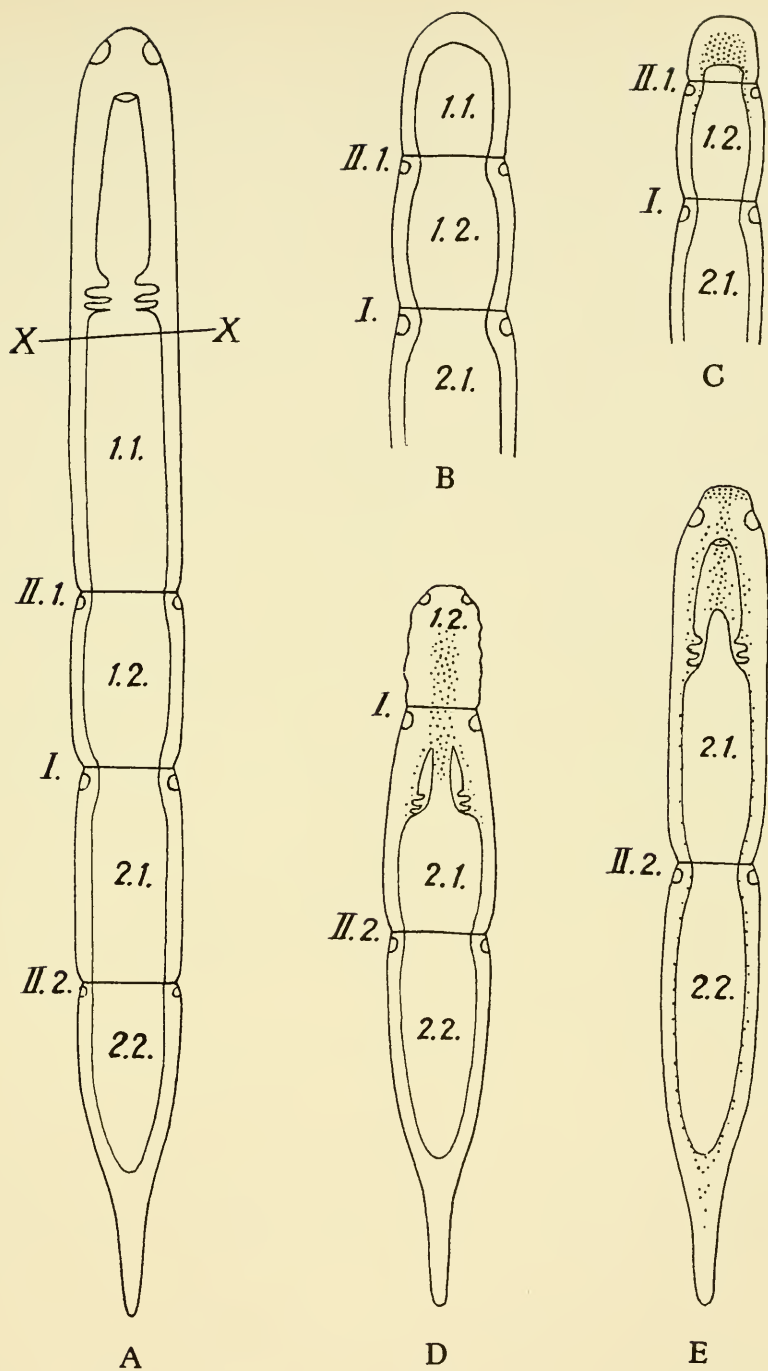


FIG. 136, A-E.—*Stenostomum grande*. Reduction and destruction of zooids resulting from alteration of dominance; zooids and fission zones numbered according to origin and order of appearance. A, chain of four zooids, sectioned at XX; B-E, stages in reduction and destruction of headless part of zooid 1.1. and of zooid 1.2. (from Child, 1903b).

anterior to it is evident from Figure 137. In Figure 138, a chain of *S. tenuicauda*, there is even more extensive destruction. After section in the anterior zooid (XX of Fig. 138, A) the posterior part of this zooid (I.I.I.I.) and the next two zooids (I.I.I.2. and I.I.2.) are progressively reduced and destroyed by zooid I.2.I. (Fig. 138, B-F), and this zooid finally remains as a single individual, fission having occurred posterior to it at fission zone I. (Fig. 138, A). This reduction and destruction of zooids occur only when their heads are in early stages or have been removed. Sometimes the headless part of an anterior zooid will reconstitute a head rapidly enough to prevent its destruction, though it may undergo some reduction before the head region attains a sufficient dominance to prevent it. The farther anterior the level of section in the anterior zooid, the more frequently does head reconstitution result; that is, the higher the gradient-level of section, the more rapidly does head reconstitution take place and the less likely is obliteration of the zooid gradient and destruction of the zooid to occur. On the other hand, an already visible reconstituting head at a cut end may finally be inhibited and destroyed like the developing head regions of younger zooids.

In the normal development of the *Stenostomum* chain each new zooid originates from the posterior region of a zooid already present; consequently, in all cases a more advanced head region is anterior to one less advanced. The older anterior component of a pair is evidently able to prevent extension of dominance anteriorly from the younger head posterior to it. When a headless zooid is anterior to a head, or an earlier zooid anterior to a more advanced head region, without an older head region still farther anterior, the dominance of the posterior head evidently extends anteriorly and obliterates or perhaps reverses the gradient, and reduction and disintegration follow.

Whether a zooid is able to maintain itself or is destroyed in these experiments probably depends chiefly on the developmental stage of its cephalic ganglia and the extent to which they have attained connection with and have brought about reorganization in, the nervous system posterior to them. Until a certain degree of nervous dominance over posterior regions is attained by a zooid, it may be destroyed by a more advanced head region posterior to it, provided there is not a more advanced head region at a still more anterior level. If these suggestions are correct, it follows that the dominance of an older head region must still be more or less effective in a zooid developing from its posterior region, at least until the head region or the ganglia of this zooid have attained a certain

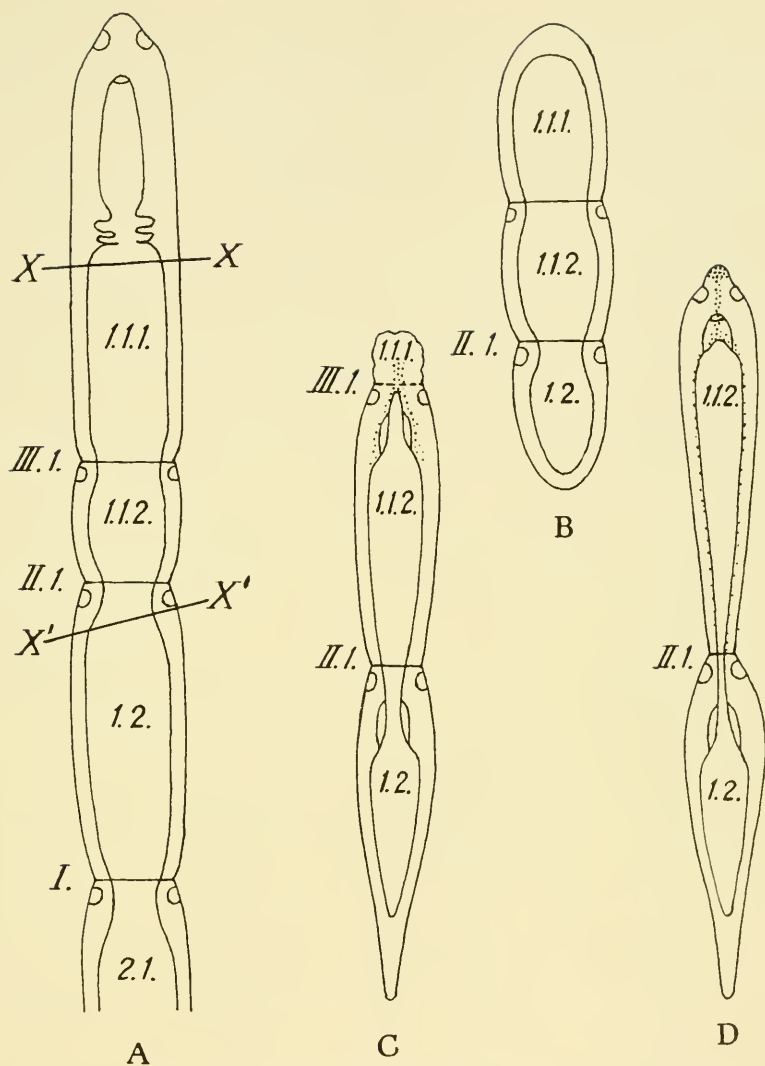


FIG. 137, A-D.—Reduction and destruction of zooids by a more advanced head region in *Stenostomum grande*. A, portion of chain between XX and X'X' used; B-D, stages of destruction; cellular material from destroyed parts in pseudocoel indicated by dots (from Child, 1903b).

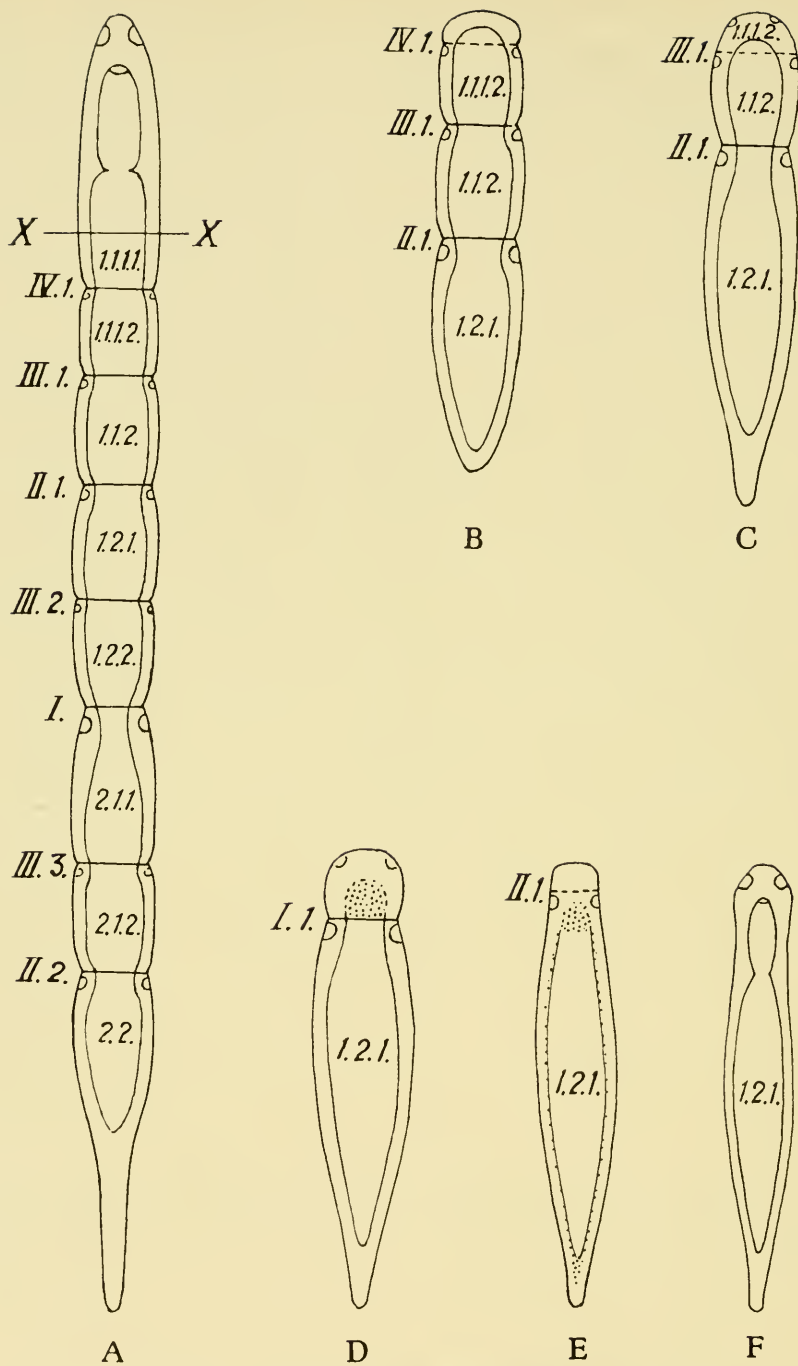


FIG. 138, A-F.—Destruction of zooids in *Stenostomum tenuicauda*. A, section at XX, fission later at zone I., still later at zone III.2.; B-F, stages of destruction (from Child, 1903b).

stage of development. And if this is the case, the physiological isolation making possible development of a new zooid is not complete isolation. That it is not complete is further indicated by the fact that contraction resulting from stimulation of an advanced head may extend posteriorly beyond an early fission zone but may be blocked at an advanced zone.

In *S. leucops* head development from an anterior cut end is very frequently completely inhibited, apparently by the rapid development of a fission zone and head at a more posterior level. This development may have begun before section but not have become visible, or may be initiated by isolation of the piece and, being more rapid than reconstitution at the anterior cut end, inhibits the latter (Van Cleave, 1929). No case of reduction and destruction of zooids or of head reconstitution from anterior cut ends was observed in more than a hundred pieces of a Japanese species resembling *S. leucops*, cut in various relations to visible fission zones; but new fission zones developed very rapidly in the pieces, and their dominance apparently inhibited reconstitution at the anterior cut end. These species differences suggest that if head reconstitution at a cut end is slow, as compared with head development at a fission zone, the latter may inhibit the former.

Destruction of zooids and inhibition of head reconstitution at a cut end in an animal in which head development occurs rapidly at a fission zone are of considerable interest as effects of obliteration or reversal of a gradient by change in position of the dominant region. In *S. grande* this reversal determines not only inhibition of development but complete loss of the epithelial character of the body layers and their separation into isolated cells. In *S. leucops* and the Japanese species the reversal of dominance has less extreme effects; it inhibits head reconstitution without destruction of zooids. The question may be raised whether the presence of a gradient is necessary for maintenance of the epithelial character of the body layers in *S. grande*. The isolated cells are not killed but have evidently lost pre-existing relations to each other. In this connection certain other suggestive cases may be recalled. When the gradient of the axis of the alga *Griffithsia* is obliterated by differential inhibition with chemical agents, the cells separate but are not necessarily killed, and with return to normal environment may reconstitute new axes (Child). With decrease or obliteration of the gradient in the blastula of *Phialidium* loss of epithelial character and immigration into the blastocoel of cells from all parts of the blastula wall, instead of from the basal region only, occur (p. 167). The entoderm of echinoderm gastrulae and exogastrulae shows

a similar loss of epithelial character with obliteration of gradient by differentially inhibiting conditions (chap. vii). However, complete reversal of gradient and dominance is possible in *Corymorpha* and many other coelenterates and in planarians without isolation of cells. Even if it should be found that a gradient is necessary for the origin of epithelial order, its persistence is evidently not always essential for maintenance of epithelial character.

EFFECTS OF OTHER PARTS ON DOMINANT REGIONS

Although a new dominant region originates independently of other parts in many plants and animals, its scale of organization and in some forms its morphological pattern may be affected by other parts. In reconstitution this effect is usually inhibition.

In pieces of *Tubularia* or *Corymorpha* stem below a certain length, but still two or three times the length of the hydranth primordium developing on longer pieces with distal end at the same level, the primordium length decreases with decrease in length of piece (Driesch, 1899; Child, 1907e). Driesch regarded this decrease as indicating a teleological relation between length of piece and primordium length. Actually, however, the primordium length decreases less rapidly than length of piece, so that, with sufficient decrease in length of piece, only a hydranth develops; and in still shorter pieces, apical partial forms (Fig. 113, A-I [p. 334]). Primordium length does not adjust to length of piece, either with the aid of an entelchy, as Driesch maintained, or in any other way; but the activation following section at the proximal end of the piece, even though not sufficient to determine a hydranth there, does determine, at least temporarily, a gradient, as dye reduction shows; and in pieces below a certain length this opposes the distal gradient and decreases scale of organization of the distal primordium somewhat. That this is the case is readily shown by delaying proximal section for different periods after distal section. Driesch's teleological assumptions are not only unnecessary but fail entirely to account for the apical partial forms.

Certain planarian species show an inhibiting action on head development originating from the posterior end in pieces below a certain length and increasing in effectiveness with decrease in length of piece at a given body-level. In the discussion of differential inhibition of the planarian head (pp. 177-90) it was pointed out that this physiological inhibiting factor originates at the posterior cut end; that it is effective only below a certain length of piece, which differs in a definite graded manner with

body-level and physiological condition of animals; and that section of the nerve is almost as effective in inhibiting the head as section across the whole body. Apparently the inhibiting factor is, at least in large part, nervous in character, a stimulation resulting from section of the nerve cords at the posterior end of the piece (Watanabe, 1935*b*) and probably from effects upon them of the activated tissue there. Since active tissue and nervous system are apparently the chief factors in dominance in the mature planarian and the inhibiting factor also appears to be nervous, the conclusion seems justified that it acts by decreasing the independence of the cells concerned in head formation.

At first glance it may seem improbable that a nervous stimulus from more posterior levels can inhibit head regeneration, but a brief analysis will show the grounds for this conclusion. It is evident that the cells at any body-level are determined and continue to function as that particular body-level only as long as they represent a certain relative gradient-level and are in physiological relation with other parts, chiefly the dominant region. This determination is reversible; and when they are isolated from dominance, every body-level can become a dominant region and give rise to a head and so determine reorganization of other parts. In other words, every body-level will develop cephalic ganglia and a head when freed from dominance and relations with other parts. Head regeneration is a self-differentiation, and nervous tissue is apparently the primary differentiation in head development. In fact, planarian parenchyma cells in tissue culture give rise to long fibers and become indistinguishable from cultures of ganglionic cells (Murray, 1931). Nervous stimuli from other parts of the body than the dominant head region probably play some part in maintaining the characteristics of a particular body-level. When a given level is isolated by section from more anterior levels, the cells adjoining the level of section tend to lose their characteristics as cells of a particular level, undergo a change in condition, and become a new dominant region. But if another section is made posterior to the first, but within a certain distance from it, the stimuli resulting from section of the nerve cords and the activation of cells adjoining the posterior cut end tend to maintain the characteristics of the body-level represented by the cells at the anterior end of the piece. They represent factors in the physiological relations of these cells with other parts and, while not sufficient to prevent head formation in the absence of posterior section, are evidently intensified by such section and become effective in partly or completely inhibiting head regeneration because their effect is to keep the cells functional parts

of the body. These stimuli apparently undergo a decrement in effectiveness with transmission anteriorly; consequently, posterior section beyond a certain distance from the anterior section has no effect on head formation. However, the decrement may be only apparent and is perhaps actually a block rather than a decrement. In pieces from anterior body-levels in which activation and development at the anterior end of a piece is more rapid than farther posteriorly, posterior section must be at a very short distance from the anterior end to be at all effective in inhibiting head development. At more posterior levels of the anterior zooid it is effective over a greater distance, perhaps because less effectively blocked by the less intense anterior activation. This relation to body-level and length of piece is shown in Figure 66 (p. 182); it is evidently an expression of differences in condition at different gradient-levels.

Demonstrative evidence that the physiological factor inhibiting head regeneration is associated with effects of posterior section is provided by the results of different periods of delay of either anterior or posterior section (Child and Watanabe, 1935a). Pieces of *Dugesia dorotocephala*, including the region *XP* (Fig. 139, *A*), develop 100 per cent normal heads; but pieces *XY*, with anterior ends at the same level but only $1/8$ or less of the body length of animals 16–20 mm. long, are almost or quite 100 per cent acephalic. If anterior section at *X* is made first, the posterior section at *Y* later, head frequency increases with increasing delay of the posterior section (Fig. 139, *B*). Even 1 or 2 hours' delay is usually sufficient to increase head frequency. With 12 hours' delay of posterior section head frequency increases from almost complete acephaly to almost 100 per cent normal, and with 24 hours delay it is 100 per cent normal.

For delay of anterior section pieces *AY* are cut, and later pieces *XY* are taken at intervals from successive lots. Figure 140 shows the change in head frequency with delay of anterior section up to 96 hours. There is usually a slight decrease in frequency with 12–24 hours' delay. Apparently not merely the posterior cut surface but the cell activity following section plays a part in inhibiting head regeneration. Comparison of Figures 139 and 140 shows that with delay of anterior section the inhibiting effect of posterior section persists over a much longer period than with delay of posterior section.

Inhibition of head development in relation to length of piece and body-level occurs in different degree in different planarian species.²² Even in

²² Cf. Child, 1913c, 1914d; Sivičkis, 1923; Buchanan, 1933; Watanabe, 1935b; Child and Watanabe, 1935a; Abeloos, 1930.

extremely short pieces of some species posterior section has little or no effect; in others there is inhibition, but less than in *D. dorotocephala*. The species differences probably result chiefly from differences in rate or intensity of anterior activation following section, in relation to the effectiveness of the inhibiting factor.

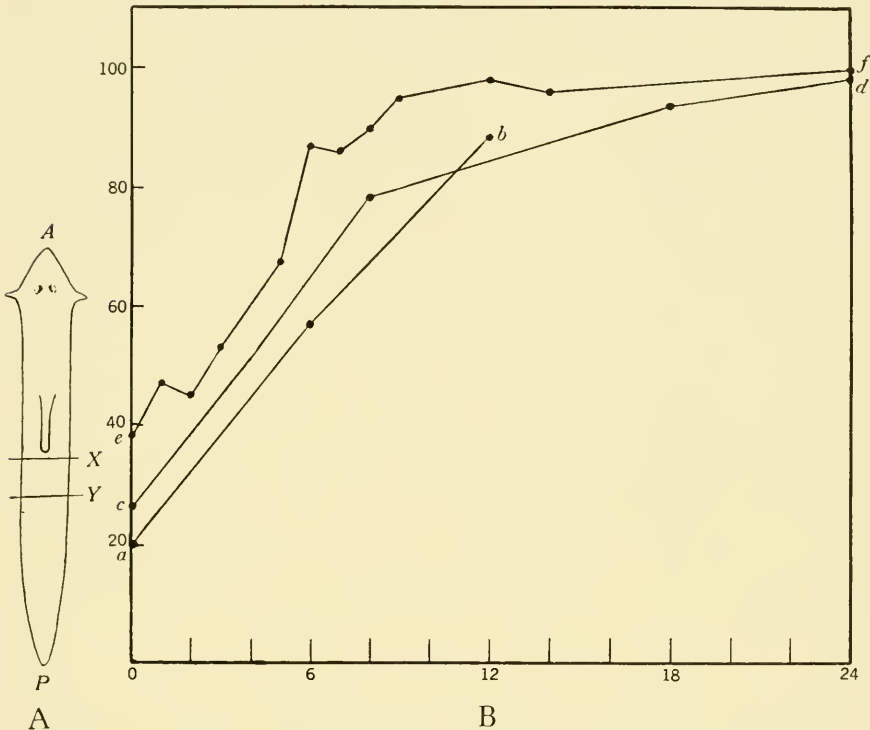


FIG. 139, A, B.—Effect of delay of posterior section on planarian head frequency (*Dugesia dorotocephala*). A, outline indicating levels of section; B, graph of increase in head frequency with delay of posterior section; ordinates, head-frequency indices (see Appendix VII), abscissae, hours of delay; curves *ab* and *cd*, twenty-five pieces for each period of delay, from data obtained by Child with different stocks; *ef*, from combined data obtained by students in laboratory class work, more than seven hundred pieces. Irregularities are chiefly due to differences in length of pieces (from Child and Watanabe, 1935a).

Head regeneration in the Dendrocoelidae and some other forms does not occur posterior to a certain body-level, irrespective of posterior section and length of piece, probably because cells of more posterior gradient-levels do not react sufficiently to isolation by section to develop a head. They are capable, however, of giving rise to posterior ends.

This inhibition of planarian head development by posterior section, like inhibition of head development and destruction of zooids in *Stenostomum*, represents an interference by a region posterior to the level of reconstitution with a dominance already present or with the attainment of the independence necessary for head formation. The decrease in length

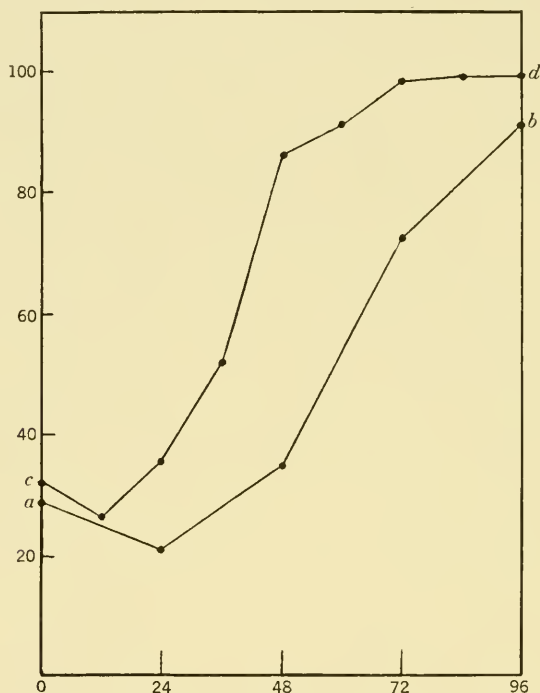


FIG. 140.—Increase in head frequency with delay of anterior section (*Dugesia dorotocephala*). Ordinates, head-frequency indices (see Appendix VII); abscissae, hours; curve *ab* from data obtained by Child with fifty pieces for each period of delay; curve *cd* from combined data of students, including more than six hundred pieces (from Child and Watanabe, 1935a).

of hydranth primordium in *Tubularia* with decrease in length of piece evidently represents a similar, but less conspicuous and effective, interference. Moreover, Hyman (1916a) has shown somewhat similar relations in *Lumbriculus*.

In the reconstitution of a new dominant region from other body-levels than the apical or anterior region there is, so to speak, a conflict between the factors concerned in the reaction initiating reconstitution and those tending toward maintenance of the original condition. The new domi-

nant region may be said to develop in spite of the isolated piece. The reconstitution of a subordinate part, such as a posterior end, apparently involves no such conflict. Nerve stimuli not only do not inhibit it but may be necessary for its occurrence, probably by their effect on metabolic level of the cells concerned. Local and temporary dominance may exist in the new posterior end, but it does not induce extensive reorganization in more anterior regions. Regeneration of appendages apparently resembles more closely posterior reconstitution than it does head reconstitution. There may be local dominance in the regenerating tissue, either distally or proximally, but it does not induce any considerable reorganization in the parts from which regeneration takes place; for example, the regenerating amphibian limb has little effect on the limb stump, except adjoining the level of section.

We have seen that new dominant regions can develop in hydroids, planarians, and annelids in the absence of other parts; and there is at present no evidence that other parts of the body, when present, contribute to the completeness of their development; they tend, rather, to inhibit it, if they have any effect. In the later course of development, more particularly in the higher animals, relations of parts may change, local patterns of dominance and subordination may arise, and parts originally developing independently of certain other parts may later be dependent on, or affected by, those parts in one way or another. For example, it has been shown by many investigators that in vertebrates absence or addition by implantation of peripheral parts in embryonic or later stages may bring about hypoplasia or hyperplasia in some part or parts of the central nervous system.²³ Such effects, of course, have nothing to do with primary developmental pattern; but they indicate that peripheral parts may acquire, after they appear, some degree of dominance over the further development of parts previously independent of them.

DOMINANCE IN COMPENSATORY REVERSALS OF ASYMMETRY

An experimentally reversible dominance of an appendage on one side of the body over an appendage of the same segment on the other side has been found in certain annelids and arthropods. The serpulid polychete *Hydroides dianthus* possesses two opercula, one highly developed and functional in closing the tube when the animal withdraws into it, the other rudimentary. Removal of the functional operculum is followed

²³ Shorey, 1909; Detwiler, 1921, 1923, 1926, 1927, 1936, particularly chap. viii and citations given there; Hamburger, 1934, 1939; Kappers, 1934; May, 1927, 1933.

by development of the rudimentary into a functional operculum, and the originally functional one regenerates as rudimentary. When only the rudimentary operculum is removed, it regenerates as rudimentary. When both are removed, both may develop the functional form or reversal, or persistence of the original asymmetry may result. Also, when the anterior part of the body is removed in the thoracic region, two opercula of functional type develop in the anterior regeneration (Zeleny, 1902, 1905*a*, 1911).

Many decapod crustacea show heterochely; that is, one chela is large, the other small; in some species only in one sex; or the two chelae differ in form and function, being distinguished as crushing and pinching or as snapping and cutting. In different species the asymmetry ranges from a high degree of uniformity as regards right-left position of the two types to approximate equality of positions. In some forms asymmetry is not reversed by removal of either claw. In others removal of the large claw is followed by development of the small into a large and by regeneration of a small in place of the original large one. In still others, like the lobster, with two claws differing in type, reversal of asymmetry does not occur in the adult but does in the young animal (Emmel, 1908); and when both claws are removed, even in the adult, both may regenerate as crushers (Emmel, 1906). Experimental reversal of asymmetry apparently has no relation to the constancy of a particular asymmetry in the species. There is no experimental reversal in some forms with approximate equality of right-left positions, or it appears only in young animals.²⁴

In the forms with experimentally reversible asymmetry the subordinate member attains dominance when the other is removed; but if both are removed at the same time, both may regenerate as dominant members, or an asymmetry may result. The experimental results are much like those with hydroid and planarian pieces. Unipolarity or bipolarity results according to the difference or similarity in condition at the two ends. So with the appendages asymmetry represents a difference in condition on the two sides of the segment concerned, and experiment shows that one side is dominant, as in unipolar forms; but if both sides begin development at the same time, both may develop as dominant but without inhibiting each other, like bipolar forms.

How the dominance of one appendage becomes effective on the other is not certainly known. Some evidence was obtained by E. B. Wilson

²⁴ For further data see Przibram, 1902, 1905, 1907, 1908, 1918; E. B. Wilson, 1903*a*; Morgan, 1904*c*, 1924; Zeleny, 1905; Emmel, 1906, 1908.

that section of one or both nerves might abolish the dominance in the crustacean *Alpheus*; but because of rapid nerve regeneration, autotomy of appendages following nerve section, and high death rate it was not regarded as demonstrative. It seems not improbable that afferent impulses from one appendage may inhibit efferent impulses to the other and so prevent its complete development, but with an equal start in regeneration neither may be able to inhibit the other. Perhaps the most interesting question in connection with these asymmetries is that of their origin in egg or embryo.

EXPERIMENTAL DETERMINATION OF PATTERN AND DOMINANCE BY ENVIRONMENTAL FACTORS

The differential effects on pattern already present of general uniform exposure to various chemical and physical environments were discussed in chapters v-vii. The present section is chiefly concerned with experimental determination and alteration of pattern by differential exposure to environmental factors.

POLARITY IN RELATION TO DIFFERENTIALS IN OXYGEN TENSION

Many hydroids are extremely susceptible to low oxygen tension in sea water. It has been shown that change from flowing to standing sea water brings about degeneration and death of hydranths and reconstitution of stolons instead of hydranths from apical as well as other regions in a number of hydroid species (pp. 172-75). Stolons can live and grow at much lower oxygen tension than hydranths. With other species, less susceptible to low oxygen in water, low concentrations of cyanide and various other inhibiting agents determine reconstitution of stolons instead of hydranths. *Plumularia* pieces in very low concentrations of cyanide and also in standing water reconstitute nothing but stolons from both distal and proximal cut ends and from cut ends of lateral branches; but in flowing, well-aerated water hydranth-stem axes develop from all cut ends. According to Barth (1938b), decrease in oxygen tension below 4.5 cc. per liter decreases markedly the rate of hydranth reconstitution in *Tubularia*, and an increase above that level increases rate and size of primordium (see also Torrey, 1912). Local removal of perisarc from pieces of *Tubularia* stem may bring about reconstitution of a single hydranth or of two with opposed polarities from the exposed coenosarc (Zwilling, 1939). In long pieces of *Tubularia* stems with proximal ends exposed to high oxygen tension, distal ends to boiled water, nitrogen, CO₂ or a mixture of 90 per cent oxygen,

or 10 per cent CO_2 all hydranths developed at the proximal ends. With short pieces results are similar but "less striking." With the same oxygen tension at both ends circulation of water at one end determined hydranth development there (J. A. Miller, 1937, 1939). *Corymorpha* resembles *Tubularia* as regards susceptibility to low oxygen. The hydranths soon die in standing water. When individuals in good condition are stained with methylene blue and placed in standing water, oxygen tension soon becomes so low about the basal regions of the crowded tentacles and about the manubrium that the dye is rapidly reduced there in well-aerated sea water with large surface open to air. In rapidly flowing water the dye is not reduced. Various attempts have been made to subject the two ends of *Corymorpha* pieces to different oxygen tensions; but by means of contraction, extension, and changes in diameter the naked stems usually make their way out of the opening in the partition between the two sea waters, or, if the opening fits tightly about the stem, separation usually results. However, other experiments with this species make it highly probable that an oxygen differential can determine which of two polarities shall develop or can actually determine polarity.

Corymorpha pieces only a few millimeters long show relatively little motility during the earlier stages of reconstitution. Such pieces lying undisturbed on the bottom of a container are freely exposed to water on one end or side, while the other is more or less closely in contact with the underlying surface and diffusion is more or less interfered with there. That this is the case as regards oxygen is readily shown by the rapid dye reduction on the surface in contact of pieces stained with methylene blue or Janus green and a gradient of decreasing rate of reduction from the surface in contact toward the free surface.

In 73 per cent of two hundred *Corymorpha* pieces 5–10 mm. long, supported on thin gauze or loose absorbent cotton near the surface of the water and lying on their sides so that both ends were equally exposed, hydranths developed on both ends. Of a like number of pieces 5 mm. long with one end, either distal or proximal, in contact with the bottom of the container, 30 per cent developed hydranths at both ends, and most of these were pieces that fell over one side in consequence of contractions and extensions. In another experiment hydranths developed at both ends in 66 per cent of fifty similar pieces frequently moved about and turned over by water currents and reversed individually. A similar lot undisturbed with one end in contact gave 22 per cent bipolar hydranths, most

of these also in pieces that failed to stand continuously on end.²⁵ In these experiments many of the unipolar forms finally developed basal ends from the ends determined as proximal by the differential exposure, whether originally proximal or distal.

In *Corymorpha* pieces lying on the side, particularly in bipolar pieces, a basal region with perisarc and stolon buds frequently develops quite independently of section from the side in contact (Fig. 141, A, B); or two

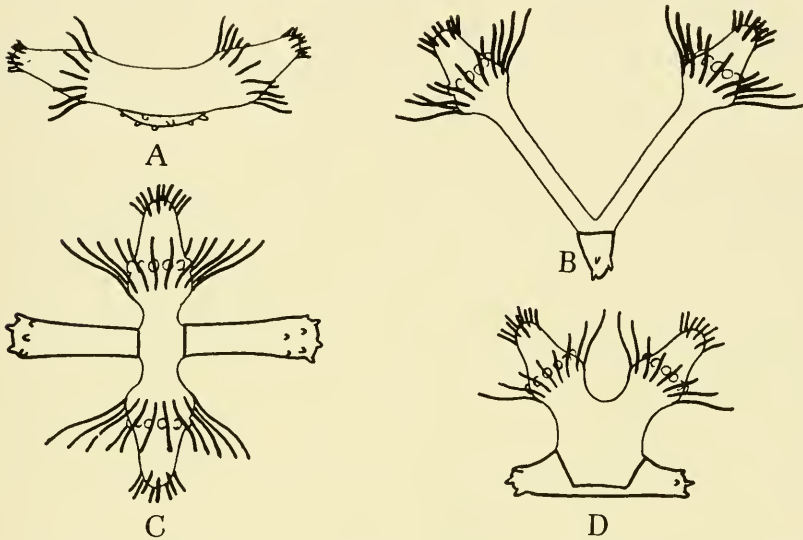


FIG. 141, A-D.—Development of bases in bipolar forms of *Corymorpha* (from Child, 1926b).

basal ends sometimes develop, producing forms of cruciate type (Fig. 141, C). The fact that these basal ends developing from lateral regions in contact are localized midway between the two hydranths of bipolar forms if these are approximately equal suggests that the two hydranth-stem gradients determine this region as the lowest gradient-level in the piece and that the differential between side in contact and free side plays a part in determining the basal region laterally instead of about the whole circum-

²⁵ The naked stems of large *Corymorpha* individuals are several millimeters in diameter when contracted, and short pieces usually remain more or less contracted for some hours after section. Reconstitution is so rapid that the hydranth primordium usually becomes directly visible within 24–30 hours; consequently, this length of time is usually sufficient to determine position of the hydranth or hydranths. Some of these experiments were reported in an earlier paper (Child, 1926b). For further discussion of hydroid reconstitution in relation to oxygen see Barth, 1940, "The process of regeneration in hydroids," *Biol. Rev.*, 15.

ference. In short pieces lying undisturbed on one side with little movement, a hydranth frequently develops from the upper, and a base from the lower, side of one or both ends (Fig. 141, *D*). Here the new gradient and axiate pattern are determined across one or both ends of the piece by the differential between upper and lower surfaces.

In all these experiments with contact-free-surface differential it is possible that accumulation of CO_2 at the surface in contact, as well as low oxygen, is concerned in determining the developmental pattern. Increase in hydrogen-ion concentration in consequence of CO_2 accumulation is probably not sufficient to have any appreciable effect. In the light of the other experiments it appears highly probable that the oxygen differential is the chief, if not the only, determining factor.

Exposure to various inhibiting agents—ethyl ether, ethyl alcohol, ethyl urethane, chloretone, and HCl -sea water (CO_2 ?)—preceding or following section or both, results, after return to water, in great increase in frequency of determination of new axes by the differential between upper and lower sides and in frequency of multiple polarities from a piece (Child, 1927*a, b*). Little or no movement occurs in such pieces after return to water until development is more or less advanced, and with sufficient exposure to the agent they often become more or less flattened on the glass or lose their characteristic structure to some extent. In Figure 142, *A* and *B*, traces of the longitudinal entodermal canals remain and make it evident that the new polarities are at right angles to the old. In *A* a hydranth develops from the upper, a base from the lower, side of each end. In Figure 142 (*B*, early, and *C*, later, stage of the same individual) the hydranth develops from the upper, the base from the lower, side, and reconstitution from the ends does not take place. In *D* the piece lost its structure and became an almost hemispherical mass, and the new axes arose as buds from its upper free surface, the lower side becoming a base and secreting perisarc. In *E* both hydranths and bases develop from the upper surface, and a large basal area from the lower. The positions of the two bases on the upper side suggest that they are determined by the dominance of some or all of the hydranths. The larger base develops between the larger hydranths, much as in many bipolar forms, and the smaller appears below the small hydranth.

The new multiple hydranth-stem axes developing after decrease or obliteration of the original gradient by differential inhibition are adventitious as regards position, that is, their localization has no definite or constant relation to a pre-existing pattern. Their general localization

on the upper surface is evidently determined by the contact-free-surface differential. Localization of any one of the multiple axes appears to be fortuitous but probably results from localization of regions of greater cell activity after return to water. With disappearance of the entodermal

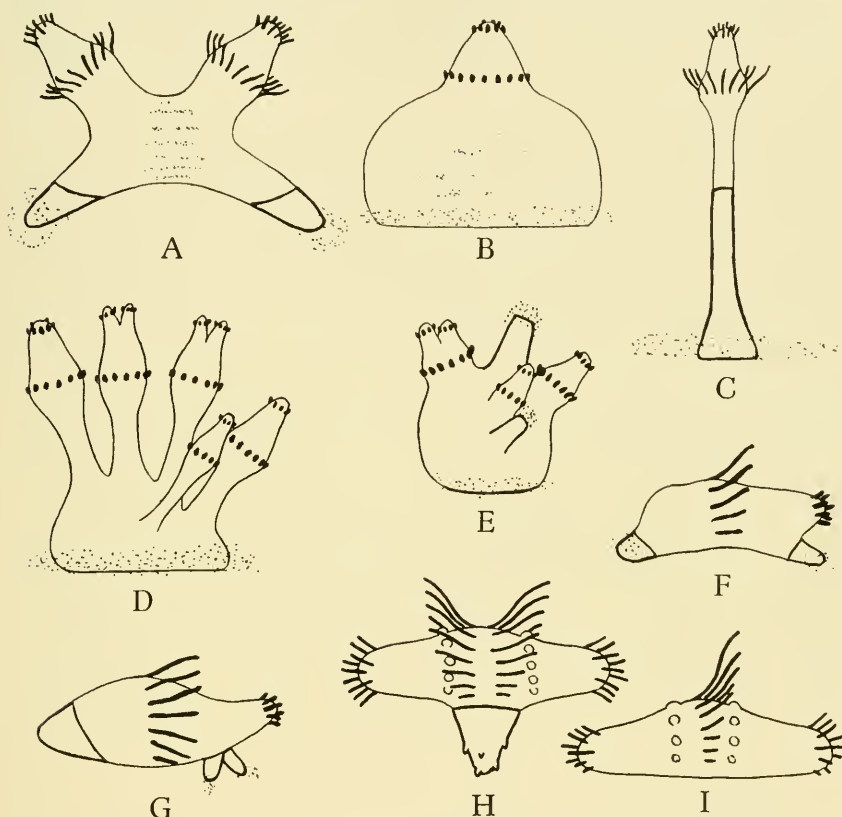


FIG. 142, A-I.—Determination of new pattern in *Corymorpha* pieces by the contact-free-surface differential after decrease or obliteration of the original polarity by differential inhibition. A-C, ethyl alcohol 2 per cent for 48 hr. following section, development in water; D, ethyl ether 0.25 per cent for 26 hr. preceding section, five new axes from upper side after return to water; E, chloretone 0.02 per cent for 26 hr. following section; F-I, dorsiventrality determined by the contact-free-surface differential after slight inhibition (from Child, 1927a).

canals their cells tend to accumulate irregularly in small groups, and there is some evidence that these cell groups just beneath the ectoderm may play a part in localizing the new axes.

Short pieces lying on one side with little or no movement or change in position in well-aerated standing water and without preceding inhibition

usually develop hydranths at one or both ends; but after temporary exposure to slightly inhibiting conditions that retard reconstitution but do not obliterate the original polarity or the proximal end as factors in determination, the hydranths often show a dorsiventrality in relation to the contact-free-surface differential. Tentacles may show a gradient in length and rate of development decreasing toward the side in contact, and one or more bases may develop from the side of the hydranth or hydranths in contact (Fig. 142, *F-I*).

It is sufficiently evident that development of bases or stolons, either in *Corymorpha* or in many other hydroids, is not a specific reaction to contact, for these parts develop under the dominance of apical regions or under somewhat inhibiting external conditions quite independently of contact. In the experiments described above, the contact is apparently merely a factor in determining low-gradient level.

Aggregation of cells into masses, following dissociation by pressing through bolting-cloth or otherwise, and development of individuals and partial forms from the aggregates have been observed in numerous sponge species and in several hydroids.²⁶ How developmental pattern originates in these aggregates is of considerable interest. They result from chance contact of cells and may differ greatly in size; and the larger aggregates may be cut into smaller ones, as desired. If an inherent persistent polarity is present in the cells, orientation to each other or to some external factor would have to be assumed to account for polarity of the whole. Actually, however, the sponge cells chiefly concerned in the development of aggregates do not appear to have a definite polarity, and the polarities of hydroid cells do not coincide with the polarity of the whole. If the cells orient to form epithelia, their orientation must be according to their polarities rather than according to a superpolarity of the whole; and if they orient to some external factor, the same difficulty arises. If polarity is primarily a gradient imposed on the aggregate, there are two possibilities of origin: it may be determined by an external differential—for example, an oxygen differential—or perhaps in some cases, at least in the hydroids, by a chance group of cells with higher metabolism than others, such as cells from the distal region of a *Corymorpha* stem. In sponge aggregates canals develop in relation to a region of greater thickness between

²⁶ For aggregation and development from dissociated cells of sponges see H. V. Wilson, 1907, 1911a, b; K. Müller, 1911; Huxley, 1921a, b; Galtsoff, 1925; De Laubenfels, 1932. Development of sponges from aggregates has also been observed by the writer in a considerable number of species (unpublished). For hydroids see H. V. Wilson, 1911b; C. W. Hargitt, 1915; Child, 1928c.

surface in contact and free surface as a center, and the osculum forms in this region on the free surface. Gravity is not concerned; probably the contact-free-surface oxygen differential is the chief factor, but demonstration seems to be lacking. Swimming sponge larvae in certain developmental stages aggregate when brought into contact, and sponges may develop from the aggregates with osculum on the free surface and a pattern without any conceivable relation to the pattern of individual larvae (H. V. Wilson, 1907).

Experiments with *Corymorpha* aggregates, consisting of cells from many individuals, indicate that the contact-free-surface differential is the chief factor in determining the polarity of the resulting individual or partial form. Aggregates remaining undisturbed with one region continuously in contact usually (79 per cent in characteristic lots) give rise to unipolar complete individuals on a small scale of organization. Aggregates of approximately the same size, moved about and turned over repeatedly at somewhat irregular intervals of one to several hours, usually (86 per cent) develop only apical parts of the manubrium on a larger scale but are mostly unipolar. Probably polarity is determined by the differential exposure in intervals between change of position. Experimental attempts to determine the time necessary for determination of polarity by the contact-free-surface differential have not yet been performed, but it is certainly not long. In the aggregates moved about at intervals the changes in position permit development on a much larger scale of organization; consequently, apical partial forms are the characteristic result (Child, 1928c). Aggregates kept free from continued contact by water currents or by continued change of position with respect to gravity by slow revolution on a vertical wheel in closed containers usually remain spherical, do not develop at all, and finally, after several days, become completely enclosed in perisarcal secretion and remain without further change, except decrease in size of the living tissue inside the perisarc, until death. However, some aggregates develop partial or complete axes, even when kept entirely free from continued contact on the revolving wheel, probably because of the presence in some region or regions of the aggregate of a higher level of cell activity, perhaps a few cells from distal stem-levels. A few such cells near together by chance may initiate a new dominance and gradient, and, if more than one such group is present, more than one axis may develop. At present there is no evidence of determination of the polarity of an aggregate by an inherent persistent polarity of its cells and their orientation to each other.

In most of the experiments described thus far in which the oxygen differential is certainly or probably a factor in determining where a hydranth or hydranths shall develop, it is primarily a regionally selective factor. In the pieces with transverse cut ends it determines which of two possible polarities shall develop; the activation following section and the resulting dominance and gradient do not depend on the oxygen differential, but they are the factors directly concerned in determining the axiate pattern of the hydranth that develops. The oxygen differential determines that this dominance and gradient at one end shall be adequate for hydranth development, and in determining the intensity of activation at the end or surface of a piece it plays a part in determining the length of the resulting gradient and the scale of organization of the hydranth, and to that extent is concerned directly in determining the polar pattern. In the aggregates of dissociated cells the contact-free-surface differential, probably chiefly or wholly an oxygen differential, may apparently be directly concerned in determining the polar gradient. However, whether the differential acts merely as a regional activator or directly as determiner, the final result is determination of the polarity of the whole piece or aggregate.

These experiments with hydroids have been discussed at some length because they appear to be particularly significant for the problems of developmental pattern and polarity. If a differential in oxygen tension can determine a physiological polarity that can be distinguished as a gradient and becomes the basis of an axiate pattern with definite differentiations along its course, the most logical and obvious conclusion is that physiological polarity in its simplest terms originates as a gradient involving differences in rate of the basal metabolism characteristic of the protoplasm concerned.

DETERMINATION OF RECONSTITUTIONAL PATTERN BY OTHER ENVIRONMENTAL FACTORS

Light has been shown to be an important factor in determining axiate pattern in many plants, but influences chiefly the course of vegetative development. In regeneration of the alga *Bryopsis*, development of a new thallus axis instead of rhizoid axis can be induced by light at the proximal cut end, although the plant has a well-defined polarity (Noll, 1900; Winkler, 1900a). This is one of the few plants in which reconstitution occurs directly from the region of injury, as it does very generally in animals. According to Loeb (1895) and Goldfarb (1906, 1910), light is necessary for reconstitution, or for continued reconstitution of hydranths in certain hydroids; but this effect is apparently on axiate patterns already

present rather than a determination of the patterns. Doubtless it does alter gradients that may be present, and perhaps with proper procedure it may determine axiate pattern.

Single cells of the red alga *Griffithsia bornetiana*, subjected to electric current, develop rhizoids toward the anode, irrespective of the original polarity; and chromatophores migrate toward the anode. This localization of rhizoid formation is regarded as resulting from migration of charged particles (Schechter, 1934).

Internodes, that is, pieces of stem between successive branches, of the hydroid *Obelia* in sea water usually give rise to hydranth-stem axes at both ends, development distally being more rapid than proximally. In this hydroid reconstitution involves outgrowth of tissue from the ends, rather than reorganization without outgrowth, as in *Tubularia* and *Corymorpha*. Electric current of proper density, flowing longitudinally, determines a high frequency of hydranth-stem development on the end toward the anode and delays or completely inhibits similar development at the end toward the cathode (Lund, 1921c). With certain current density, hydranth development is inhibited at the cathodal ends of *Tubularia* pieces, as reported by Lund for *Obelia*; but with a sufficient increase in current density the inhibition is reversed and becomes greatest at the anodal end. In *Eudendrium* pieces inhibition is greatest at the cathode end, but in *Pennaria* pieces it is greatest at the anodal end with all current densities used (Barth, 1934a). According to Barth, effect of current in these cases appears to be largely a difference in degree of inhibition at the two ends, although in *Tubularia* pieces hydranth frequency is higher at the anodal end in current densities which determine cathodal inhibition than in controls. This, however, may result from the cathodal inhibition, for it has been repeatedly shown that hydranths develop more rapidly at proximal ends of pieces when distal hydranth development is inhibited.

Strictly speaking, electric current, like the oxygen differential, determines in these cases which of two possible polarities shall express itself in development. The activation following section determines the polarity or polarities in the piece, and the current obviously affects the activation at cathode and anode differentially or in different degree. The result is, of course, determination of polarity of the piece, but perhaps the current should be regarded as selecting the polarity rather than as determining it. Whether electric current can determine a new polarity in hydroid tissue independently of a cut end has not yet been discovered, but that it can do so seems highly probable. Undoubtedly, a sufficient difference in many

other physical or chemical factors at the two ends of *Tubularia* or *Corymorpha* pieces would have effects very similar to those of electric current; but, except for temperature difference, reported to be effective in *Corymorpha* by Gilchrist and Schmidt (1932) and in *Tubularia* by J. A. Miller (1939), and for difference in H-ion concentration, also stated to be effective,²⁷ experimental evidence seems to be lacking.

In many plants gravity is a factor in localizing regions from which rhizoids or roots develop and in determining what bud primordia shall develop, but apparently it is not an essential factor in determining the root or bud gradient. Its effect in these cases appears to be determination in certain regions of conditions favorable for initiation of rhizoid or root formation. Such determination may, of course, play a part in establishing a general polarity of the whole plant or piece, but the polarity of each rhizoid or root apparently results from the local activation and initiation by it of a gradient system. In buds activated by gravity the gradient pattern is already present, and gravity appears to be merely an activator. Gravity is supposed to bring about differential distribution of substances of different specific gravity and may determine gradients in concentration of such substances, but these gradients are not essential to the axiate patterns of particular rhizoids, roots, or buds.

Prolonged low-speed centrifuging of isolated cells of the alga *Griffithsia* tends to localize development of new apical cells and resulting thallus axes centrifugally, where heavier substances are concentrated; but the concentration of substance is regarded as stimulating or activating rather than as directly determining the new polarity (Schechter, 1935).

According to Loeb (1891), pieces of the hydroid *Antennularia antennina*, suspended in various positions, develop stolons from parts toward the earth, hydranth-stem axes from parts extending in the opposite direction. Stolons develop even from the apical ends of lateral branches or of the main axis when these point downward. Further experiments by Morgan (1901) and Stevens (1902, 1910) do not entirely confirm Loeb's results. These authors find that stolons often develop from both ends of suspended pieces and that pieces in various positions on a slowly revolving vertical wheel in water usually develop hydranth-stem axes. As in the case of *Plumularia*, this latter result is probably due to better oxygen supply and removal of CO₂ with the constant change of position on the

²⁷ According to Komori (1933), pieces of *Tubularia* reconstitute stolons or nothing from the distal ends at pH 6 and hydranths from proximal ends at pH 8.45. This difference in H-ion concentration is not great and raises the question whether hydrogen ion or CO₂ is the effective factor in this case.

revolving wheel. That Loeb's results may have been due to an oxygen gradient decreasing downward is perhaps possible. In general, gravity or, as will appear below, even high-speed centrifuging does not seem to be a very important factor in determining or altering axiate pattern in animals, though there are some cases in which one or the other factor is effective; and further experiment, particularly with the very high centrifuge speeds now possible, may bring new evidence on this point.

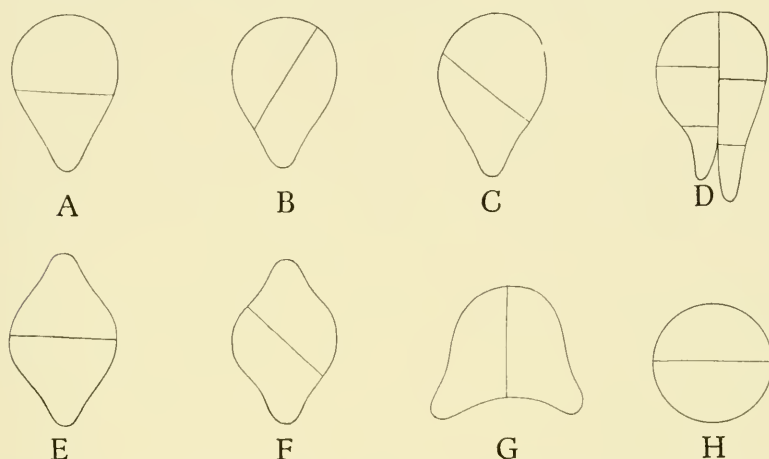


FIG. 143, A-H.—Early development of *Fucus*. A, normal development with plane of cell division at right angle to axis indicated by rhizoid outgrowth, separating a rhizoid cell and a thallus cell; B-D, altered relations of rhizoid outgrowth and division plane resulting from change in direction of illumination; E-H, bipolar forms resulting from periodic change of 180° with respect to direction of light.

EXPERIMENTAL ALTERATIONS OF EMBRYONIC AND AGAMIC PATTERN BY ENVIRONMENTAL FACTORS

So far as data are available, pattern in most eggs and early embryonic stages appears to be relatively stable in relation to external differentials, but in some forms new pattern can be determined experimentally by such factors. Perhaps the most interesting case is the egg of the alga *Fucus*. This egg apparently possesses a polarity when shed, since it can develop the axiate rhizoid and thallus in complete darkness without any evident relation to external factors. The first indication of development in the originally spherical cell is a bulging or outgrowth from a part of its surface, followed by a cell division in a plane transverse to the axis of the outgrowth (Fig. 143, A). The outgrowth represents an early stage of the first rhizoid and, with various methods, shows a gradient with high end

at the tip. The other cell gives rise to the thallus; and, as this develops, a gradient, with high end apical gradually appears in it. It has long been known that polarity of this egg can be determined by light, that is, by differential illumination, the rhizoid developing on the side away from the light, with the first division plane transverse to the direction of illumination.²⁸ More recently a certain range of wave length toward the blue-violet has been shown to be chiefly effective (Hurd, 1919, 1920). With repeated (e.g., hourly) change of position with respect to direction of incident sunlight, the axis indicated by outgrowth of rhizoid and the division plane may form any angle with each other (Fig. 143, *B-D*). From changes of 180° in position bipolar forms frequently result, either with rhizoids at or near opposite poles (Fig. 143, *E-G*) or with equal division and no rhizoids, like Figure 143, *H* (Child, unpublished). These variations suggest that rhizoid outgrowth and cell division require different periods or intensities of illumination for determination of the usual relation, but further experiment on this point is desirable.

Polarity of this egg may also be determined by electric current, the rhizoid developing on the side toward the anode (Lund, 1923*b*). When several eggs lie close together, there is mutual determination of polarity, the rhizoids arising toward the center of the group, thus indicating a chemical differential dependent on presence of the eggs. It has been further shown that increase in H-ion concentration increases this group effect and that a H-ion gradient induces rhizoid development on the more acid side, up to a certain concentration, and above that on the less acid side. However, even with removal of excess CO₂ from acidified sea water the possibility that it is concerned in this effect is not entirely excluded.²⁹ In another line of experiment it was found that the rhizoid tends to form on that side of the egg to which a sufficient concentration of the potassium salt of indole acetic acid (heteroauxin) is applied, and in the light of these results it was suggested that certain agents which determine polarity in this egg may do so by influencing in some way the activity of auxin in the egg.³⁰ Whitaker points out that this hypothesis will account for most effects of increased H-ion concentration, since acidity increases auxin activity. On this basis effects of high H-ion concentration result from increase in auxin activity to the point at which it becomes inhibitory.

²⁸ Farmer and Williams, 1898; Winkler, 1900*b*; Küster, 1906; Kniep, 1907. The first division plane and the polarity of the *Equisetum* spore show a similar relation to direction of incident light (Stahl, 1885).

²⁹ Whitaker, 1935, 1937*a*, 1938*a*; Whitaker and Lowrance, 1937.

³⁰ Du Buy and Olson, 1937; Olson and Du Buy, 1937.

Polarity of *Fucus* eggs may also be determined by centrifugal force. After a certain intensity and period of centrifuging eggs suspended in firm agar-sea water to prevent possible orientation, 93-99 per cent of eggs with persisting stratification develop rhizoids at or near the centrifugal pole, but redistribution of egg substances abolishes this effect (Whitaker, 1937*b*). According to Beams (1937), ultra-centrifuging does not affect polarity of eggs of another species of *Fucus*. Whitaker (1938*b*) finds, however, that at pH 7.9-8.1 the H-ion concentration of sea water, all ultra-centrifuged eggs form rhizoids on the centrifugal halves, while at pH 5.8-6.0, well on the acid side, 90 per cent form rhizoids on centripetal halves. He makes the further suggestion that auxin may be adsorbed on heavy substances and attain inhibitory concentrations at the centrifugal pole in acid sea water, or its transport may be affected by amphoteric substances, with reversal when the isoelectric point is passed. Eggs exposed to a slight temperature gradient develop rhizoids on the warmer side (Lowrance, 1937*a, b*). When recently fertilized eggs are drawn into a capillary pipette with lumen small enough to elongate the egg, the pectin or cellulose wall "sets" and the egg retains ovoid form after extrusion: in darkness 96 per cent (of 114 eggs) form rhizoids at or near one end of the longitudinal axis. At pH 6, however, rhizoids tend to form on the surface in contact, where diffusion is less rapid, and this differential supersedes the effect of shape (Whitaker, 1938*c*, 1940).³¹ Whatever the physiological factors concerned, it is evident that, even though a polarity may be originally present in the egg, the polar pattern can be reconstituted by various external differentials, entirely without section or other injury to the egg.

In many animal eggs cytoplasmic differentiation is apparently so far advanced at the beginning of embryonic development that axiate pattern appears highly stable, but alteration is still possible in some eggs. When polarity is obliterated by differential inhibition in the blastula of the hydromedusa *Phialidium* (pp. 168-69), the blastula becomes solid and spherical, and after return to water rolls about on the bottom of the container for perhaps several days, ciliary activity being no longer co-ordi-

³¹ For determination of dorsiventrality in an echinoderm egg in a similar way and the interpretation suggested see p. 427. More recent papers on experimental determination of polarity in *Fucus* and the related form, *Pelvetia*, are as follows: Whitaker, 1940, "The effects of ultra-centrifuging and of pH on the development of *Fucus* eggs," *Jour. Cell. Comp. Physiol.*, 15; Whitaker and Lowrance, 1940, "The effect of alkalinity upon mutual influences determining the developmental axis in *Fucus* eggs," *Biol. Bull.*, 78; Lowrance and Whitaker, 1940, "Determination of polarity in *Pelvetia* eggs by centrifuging," *Growth*, 4.

nated. It gradually comes to rest, apparently in any position, there being no evidence of orientation when it is rolled about passively. After coming to rest, it flattens to a more or less hemispherical mass and secretes perisarc about itself (Fig. 144, *A*). With further recovery a hydranth-stem axis may develop from its upper surface (Fig. 144, *B*); or two, three, or even four new axes, one a hydranth-stem axis, the others stolons (Fig. 144, *C*), or all stolon axes (Fig. 144, *D*) may develop. These stolon axes often give rise later to hydranth-stem axes from their upper surfaces (Child, 1925*b*). The contact-free-surface differential is evidently a factor in determining the new patterns: hydranth-stem axes always develop from



FIG. 144, *A-D*.—Development of new polarities in blastula of the hydromedusa *Phialidium* after obliteration of polarity by differential inhibition. *A*, solid blastula flattened on the substrate after cessation of ciliary activity; *B*, hydranth-stem axis developing from upper surface; *C*, hydranth-stem axis from upper surface and three stolon axes below; *D*, three stolon axes (from Child, 1925*c*).

the upper free surfaces of the masses, and stolon axes always in contact with the substrate. Particular stolon axes are probably localized by slight chance differences in condition in different regions, though perhaps some unrecognized external differential may contribute to their localization. In development from planulae under natural conditions stolon axes do not appear until considerably later stages, after the hydranth-stem axes has developed. Differentially inhibited planulae give very similar results, though their polarities are not usually completely obliterated, and stolon axes develop from one or both ends but may appear elsewhere.

Polarity of animal eggs is usually not altered by centrifuging, though position of polar-body formation may be altered by displacement of maturation spindle or of nucleus, but ventrodorsality apparently can be altered by centrifugal force in some eggs. In eggs of certain sea urchins,

centrifuged before or after fertilization, the centrifugal region tends to become ventral.³² According to Lindahl, however, in centripetal fragments of these eggs the ventral side is centripetal, and in another species he finds the ventral side centripetal. Also, in eggs stretched by being drawn into a capillary tube the end in advance becomes ventral, because greater stretching of this end renders plasma colloids less stable. Deep staining of this end with Nile blue sulphate makes it dorsal. Lindahl suggests that the ventral region is an inductor and has a higher metabolism than the dorsal region and that the region where protoplasmic inclusions are aggregated becomes ventral because the inclusions determine higher metabolism in it. Differential dye reduction and susceptibility agree in indicating or suggesting higher metabolism in the ventral region in normal sea-urchin embryos.³³ But why the ventral region should be centrifugal in the whole egg and centripetal in a centripetal piece is not entirely clear.

Unfertilized *Dendraster* eggs centrifuged 6–8 minutes at 45,000 times gravity develop into plutei with an undivided centripetal lobe, situated ventrally at the angle between ventral and anal surfaces in 93.6 per cent of the larvae. Of these, 56.9 per cent have the lobe lateral on the ventral surface, in 14.9 per cent it is nearly median, in 22 per cent intermediate (Pease, 1939). Ventrodorsality is believed to be present in the egg and to be shifted by centrifuging. A “ventral-determinant” gradient is postulated with highest concentration or activity ventral, probably cortical, perhaps an enzyme, requiring a substrate probably diffuse in the entoplasm but concentrated centripetally by centrifuging and so partially rotating the ventrodorsal axis. In centrifuged eggs of the geophyrean *Urechis* the centripetal region also tends to be ventral and without relation to point of sperm entrance or first cleavage (Pease, 1938). The “determinate” cleavage pattern of ultra-centrifuged eggs of the pelecypod *Cumingia* and the polychete *Chaetopterus* is related to the stratification, though with wide variation in *Cumingia*; and polarity and bilaterality are apparently determined in relation to the cleavage pattern.³⁴

Eggs of the sea urchin *Arbacia* suspended in sugar solution of proper density can be separated by strong centrifuging into two parts, the centripetal part colorless, the centrifugal granular and pigmented; and each of these parts can be again separated by further centrifuging. These egg

³² Runnström, 1925c, 1926a; Lindahl, 1936.

³³ See pp. 134–38 and chap. vi.

³⁴ Pease, 1940, “The influence of centrifugal force on the bilateral determination and the polar axis of *Cumingia* and *Chaetopterus* eggs,” *Jour. Exp. Zool.*, 84.

fragments can be fertilized and show more or less development, the colorless halves becoming plutei, the pigmented halves containing only the male nucleus, sometimes forming blastulae and occasionally plutei. Unfertilized centrifuged halves without nucleus, when artificially activated, may cleave and form blastulae. Centripetal halves, artificially activated, usually develop normally. When centrifuged after fertilization, centripetal halves, containing both nuclei, develop, but plutei are not normal; centrifugal halves do not develop.³⁵ Evidently there is extensive reconstitution in those fragments which develop, but exactly how the pattern is altered is not known. These, like many other centrifuge experiments, suggest that the pattern in these eggs is chiefly or primarily cortical but that protoplasmic content inside the cortex, as distinguished from granular inclusions, is perhaps of significance in maintaining sufficient cortical activity for development.

In most animal eggs a considerable degree of stratification by centrifuging may occur without essential alteration of developmental pattern, though, as noted above, it may alter position of polar-body formation by displacing the nucleus or maturation spindle. However, eggs of certain ascidians stratified by centrifuging show dislocation of pattern of tissues and organs (Conklin, 1931).

Perhaps the most interesting, certainly the most extensively investigated and discussed, case of alteration of animal developmental pattern by gravity and centrifugal force is that of the amphibian egg and early embryo. Only brief consideration of some of the more important points is attempted here. In amphibian eggs and early cleavage stages, maintained in inverted or partly inverted positions with respect to gravity, or centrifuged in these positions, more or less complete reversal in position of heavier and lighter substances occurs. The heavier yolk accumulates in the original apical region, displacing the lighter parts of the cytoplasm to the upper, originally basal region, and certain alterations of pattern result.³⁶

Development of double monsters from the inverted two-cell stage, first observed by Schultze, was usually interpreted as the result of decrease

³⁵ Harvey, 1932, 1936, 1939.

³⁶ Born, 1885; Schultze, 1894; Wetzel, 1895, 1896; Chiarugi, 1898; Tonkoff, 1900, 1904; Bagini, 1923; Schleip und Penners, 1925, 1926; Wiegmann, 1926, 1927; Hämmerling, 1927; Penners und Schleip, 1928; Penners, 1929; Wittmann, 1929; Dalcq et Pasteels, 1938; Pasteels, 1938; also, omitted from bibliography, Pasteels, 1938, "Recherches sur les facteurs initiaux de la morphogénèse chez les Amphibiens Anoures, I," *Arch. Biol.*, 49. For a general discussion with bibliography see Schleip, 1929, pp. 584-90, 696-715.

or elimination by the cytoplasmic movements of relations between the two blastomeres, so that reconstitution resulted in a more or less complete, instead of a half-embryo, from each. Later experiments showed that this interpretation was not adequate, for it was found that double embryos might develop from inverted undivided eggs, from four-cell, and even from eight-cell stages, and occasionally triple monsters developed from two-cell stages (Wetzel, 1896; Schleip und Penners, 1925). Most authors are agreed that the region of the gray crescent, which normally becomes the dorsal lip of the blastopore, the "organizer" region, does not change its position in inverted eggs; and, according to Penners and Schleip (1928), sinking of the white yolk occurs in different ways in different eggs, and portions of it may remain in the original basal region or at the surface along the cleavage furrows present. These authors maintain that gastrulation and organization are not necessarily dependent on the original dorsal lip region but may result from localization of such regions where a band or mass of white yolk is in contact with other cytoplasm. Double embryos arise by localization of dorsal lip regions and invagination in opposition directions on both sides of a band or streak of yolk, left behind as the yolk sank. Gastrulation and organization may also be localized in relation to other yolk masses that failed to sink or reached the surface at the lower, originally apical, pole. Evidently the region of the gray crescent is not definitively determined as the only dorsal lip region and organizer up to four-cell and eight-cell stages. Other regions are capable of becoming organizers and of determining gastrulation and position and axial direction of the neural plate. Localization of gastrulation and neural plate are related to gravity or centrifugal force only in so far as these agents alter local relations of yolk masses and cytoplasm.

Further analysis by means of partial inversion confirms the work of Penners and Schleip as regards the significance of contact of cytoplasm and yolk masses for localization of invagination and of the neural inductor or organizer and leads to the further conclusion that localization of the blastopore or blastopores is as near the original prospective dorsal lip as the relations of cytoplasm and yolk permit (Pasteels, 1938; Dalcq et Pasteels, 1938). In frog eggs maintained in inverted position by compression between slides a blastopore lip forms at the edge of peripheral yolk but, because of the compression, does not invaginate, fades out in a few hours, and another or others may be formed (Pasteels, 1939). Dalcq and Pasteels conclude that the essential pattern of amphibian development is a primary apicobasal cytoplasm-yolk gradient and a dorsal cor-

tical area with gradient of potency for gastrulation and inductor development decreasing from the mid-dorsal region of the gray crescent in the frog egg, that is, from the region of primary invagination in normal development. They regard the metabolisms resulting from, and determined by, local concentrations of cytoplasm and yolk and from relative differences in amounts of each as essential factors in development. In the single, double, or triple embryos and monsters developing from these inverted eggs polarity, symmetry—in fact, the whole axiate pattern—may be the result of a reconstitution and entirely a new pattern; but the localization of new dorsal lips, blastopores, and neural plates shows a relation to the original dorsal region, suggesting a graded differential in that region. It is of some interest to note that results of experiments on differential susceptibility, differential dye reduction, and distribution of SH-proteins and some of the data on oxygen consumption are in general agreement with these conclusions (see. pp. 151–58).

As regards the vegetative reproductions of parts in plants, it was pointed out above that external factors may determine the regions in which certain parts develop, but not their axiate patterns. It has long been known that pattern of branching in the thalli of certain algae can be determined by direction of illumination in relation to the main axis of the thallus. Branching is radially symmetrical when illumination is equal on all sides, bilateral or dorsiventral when it is unequal (Berthold, 1882). Other external factors—for example, gravity—may affect pattern of arrangement of axes in many plants though not determining the pattern of the particular axiate parts. In various plants, however, external factors may determine dorsiventrality directly. For example, it is well known that light can determine dorsiventrality in certain algae, mosses and liverworts, and in the prothallia of ferns. In some of these forms a dorsiventrality once determined can be reversed in the later growth by reversing direction of illumination; in others it is stable. Rhizoids and, in fern prothallia, sex organs develop from the side regarded as ventral, and in many liverworts the thallus itself becomes dorsiventrally differentiated. It has been shown for many of these forms that the entire dorsiventral pattern of further growth is experimentally reversible by reversal of illumination. The gemmae of various liverworts possess bipolar pattern with apical growing cell at each tip and each gives rise to two thalli that grow in opposite directions and finally become separated. Extensive experiments with gemmae of *Marchantia* and *Lunularia* have shown that their dorsiventrality can be determined by light, by gravity, or by a

nutritive agar, or even a purely mineral substrate. In general, the less illuminated side or the side toward the earth becomes ventral, but the side toward the substrate becomes dorsal. In *Marchantia*, however, the effect of light depends on temperature. With germination at high temperature the less illuminated side tends to become dorsal instead of ventral; also, with light and gravity acting in opposite directions, the effect of gravity tends to overbalance that of light at high temperature. With equal action of external factors in opposite directions on the two sides both may differentiate in the same way (Fitting, 1935, 1937). In some of the higher plants dorsiventrality may be determined by light, and gravity may also be a factor. It is an interesting question how the region of the circumference of the plant vegetative tip where the first lateral bud primordium develops, or the first two, three, or more in forms with opposite or whorled lateral buds, are determined. Perhaps a more complete acquaintance with botanical literature would show that this question has been answered, but essentially similar questions regarding animal development await an answer.

Physiological, rather than external, factors are usually concerned in localizing agamic reproductions of new individuals and development of parts and organs in animals, but there are some cases in which external factors may be concerned and regarding which the question raised above, concerning lateral bud primordia in plants, must be asked. For example, how is the region of the circumference determined where the first lateral bud appears on a *Hydra* individual? The same question arises with respect to the first medusa bud on the manubrium of *Pennaria* or other hydroids. In development of the hydranth from the planula of *Corymorpha* a single tentacle often appears before others. How is its localization on the circumference of the planula determined? This planula does not swim but creeps on the substrate with one side in contact; the first tentacle often develops on the upper side, but that it always does is not certain. In other individuals two tentacles on opposite sides, or three, equidistant from each other, develop apparently simultaneously. The apical region of the planula often turns away from the substrate before tentacles develop; but even if equal exposure determines simultaneous development of two or three tentacles, the question of how they are localized on the circumference remains. With localization of one *Hydra* bud, medusa bud, or tentacle bud, its local dominance may play a part in determining localization of another by determining that another cannot develop within a certain distance from itself. A question of somewhat different character

is that of the factor or factors determining development in one plane of the sea fans (Gorgonacea). In fans developing on more or less vertical rock faces with water movement chiefly vertical, the plane of development is usually, if not always, vertical. These and many other cases of spatial pattern and order of zooids or parts present problems of fundamental significance for our conception of development.

CONCLUSION

Some of the ways in which axiate patterns can be experimentally determined have been considered in this chapter. So far as the examples given have been analyzed, they appear to involve alteration, obliteration, and determination of dominance and a gradient or gradients as the earliest distinguishable feature of the change in pattern. In the light of the experimental data it appears that a factor operative in the reorganization of other parts in reconstitution is associated with the high end of a gradient or gradient system. In isolated pieces the region or regions most intensely activated following section or otherwise become more or less dominant and alter a pre-existing gradient or determine a new one in a different direction from that already present. A new gradient and dominance may also be determined by environmental differentials or gradients of various kinds. However a gradient is initiated, the specific constitution and physiological condition of the protoplasm concerned are undoubtedly the chief factors determining its physiological characteristics, its length, steepness, and the effective range of dominance.

The determination of polarity in *Corymorpha* cell aggregates by the contact-free-surface gradient, probably an oxygen gradient in the absence of any cut surface, the effectiveness of ganglionic planarian grafts in induction of reorganization in the host body, the obliteration of fission zones by reconstitution of heads a short distance anterior to them, the destruction of headless parts of zooids and whole zooids in early stages by a more advanced head region posterior to them in *Stenostomum* pieces, and in general the very evident relation between dominance and dynamic, rather than structural, conditions—all support the view that dominance and the gradient or gradient system associated with it are primarily dynamic in character, not structural, except in so far as activity and structure of some sort cannot be dissociated in living protoplasms. Vital activity, metabolism, appears to be the primary factor determining pattern rather than a pre-existing structure determining metabolism.

Reconstitution of a hydranth or head is not a replacement or restitu-

tion of a part removed but the development of a new axiate pattern, beginning with the apical region or head, which develops from the high region of the gradient determined by section and isolation. Reconstitution of hydranth or head at the distal or anterior end of a piece from any body-level except one immediately adjoining the original hydranth or head is just as truly a heteromorphosis as a hydranth or head developing at the proximal or posterior end. The one is "out of place" as much as the other. The parts normally present between such a developing hydranth or head and the level of section where it develops are absent until later, when reorganization is induced by the new dominance and gradient. At either end of the piece or elsewhere the reconstituting hydranth or head represents beginnings of a new axiate pattern.

Development of hydranth or head on an isolated piece can occur only with a certain degree of physiological isolation of the cells concerned from other parts of the piece; this isolation results from the activation of cells following section and isolation; this, in turn, alters or obliterates the old polar gradient pattern and determines a new one. It is no exaggeration to say that development of a hydranth or head or, in general, determination of a new gradient in reconstitution occurs in spite of the rest of the piece, that is, in spite of the pre-existing gradient and organization. The old pattern is more or less completely made over. On the other hand, development of a basal or posterior end is primarily development of a more or less subordinate part, determined either by the dominance of parts anterior or distal to it or by inhibiting external conditions. The hydroid stolon is an axiate pattern with its own dominance and gradient, but still in some degree under the dominance of the hydranth-stem axis or directly determined an external inhibiting factor.

New pattern can be determined not only by the activation following section and isolation but by implants and by external differentials—light, gravity, centrifugal force, electric current, temperature, an oxygen differential, H-ion concentration—in many organisms, both plant and animal, by more than one of these factors. Even change of shape, probably involving local or regional stretching of the cortex and consequent alteration of its physiological condition, is effective in some eggs. But however the new pattern is initiated, it is, of course, the specific constitution of the protoplasm in which it appears that determines its characteristics as developmental pattern. Induction by a dominant region of reconstitution in other parts is very generally characteristic of reconstitutive development, but a new gradient may be directly determined by

an external gradient; in those cases dominance probably develops secondarily from the high region. It appears sufficiently evident that physiological and external factors effective in determining new developmental pattern are, in general, factors influencing the physiological activity, the metabolism, of living protoplasm. Usually they are activating factors, though in some cases pattern of one kind can be transformed into another by depressing or inhibiting factors—for example, the transformation of hydranth-stem axes into stolon axes by low oxygen tension or by inhibiting chemical agents. In view of all the evidence developmental pattern appears to be primarily an expression of the dynamics of living protoplasms.

CHAPTER XII

INDUCTORS AND SO-CALLED "ORGANIZERS" IN EMBRYONIC DEVELOPMENT

THE demonstration by Spemann and his co-workers that the region of the urodele amphibian embryo which becomes the dorsal lip of the blastopore and on invagination forms chorda-mesoderm is a dominant region and can induce or determine development of other parts provided experimental evidence in support of the conclusion that a relation of dominance and subordination may be of fundamental significance in the development of the amphibian embryo. Such a relation had already been shown to exist for certain organs of later developmental stages—for example, in induction of a lens by an optic vesicle or cup. Dominance of certain parts and physiological or physical isolation from that dominance had also been shown to be fundamental factors in agamic and reconstitucional development (see chaps. ix–xi). However, the discovery of a regional dominance and of the presence of a so-called "organizer" or "organization center" in amphibian development has exercised a sort of dominance over the field of experimental embryology and has resulted, during the last fifteen years, in a tremendous amount of investigation on various aspects of the problem and in the discovery or postulation of many other "organizers," concerned with one feature or another of development, not only in amphibia but in many other organisms. In chapters ix–xi it was shown that dominant regions in many reconstitutions and agamic reproductions are apparently primarily the high regions of gradients and that their dominance results from their activity rather than from specific differentiation. Dominant regions resulting from section and isolation alter existing gradients or determine new ones. In view of the evidence concerning the relation of gradients and dominance to axiate pattern, it appears probable that the gradient determined by an activated region is the real organizing factor. According to this conception, the region of primary activation is an organizer only indirectly, by initiating and determining a gradient pattern; conditions at different levels of this pattern determine the orderly localization of parts along an axis. In short, these experimentally determined dominant regions in the

simpler animals are inductors of gradients, and the gradients are the real organizers. Since the concepts of inductors and organizers have developed from experiment on embryonic stages, it is necessary to raise the question whether, or to what extent, similar factors are concerned in the action of embryonic inductors and the inductors concerned in reconstititional development of isolated parts of adult animals. Experimental data bearing directly on this question from the embryonic side concern chiefly certain echinoderms, fishes, amphibia, and birds; but embryonic development of various other forms affords some data which are also suggestive or positive.

INDUCTION IN ECHINODERMS

Before turning to the experimental data attention is again called to a few points in the earlier discussions of echinoderm developmental pattern (chap. vii). The hypothesis of two opposed, overlapping gradients of concentration of substances, an "animal" and a "vegetal" gradient, the one decreasing basipetally, the other acropetally, advanced by Runnström and accepted and developed by his co-workers, Hörstadius and Lindahl, has been discussed (pp. 241, 243). A third, ventrodorsal gradient has also been postulated, and Hörstadius has suggested two opposed gradients in the right-left axis.¹ Since animal and vegetal gradients overlap according to this hypothesis, they must be specifically different; Runnström (1933) and Lindahl (1936) have advanced further hypotheses concerning the character of animal and vegetal metabolisms. Moreover, to account for their experimental results these authors find it necessary to assume that one of these gradients in the polar axis may "suppress" the other more or less completely, that both may be altered in extent or otherwise, and that certain external agents affect one or the other specifically.

Von Ubisch has arrived at somewhat different conclusions.² He also postulates existence of two substances, ectodermal and entodermal, de-

¹ References p. 241, footnote 20. In discussion of the experiments of these authors it will be convenient to follow their usage of the terms "animal" and "vegetative," except that "vegetal" is used instead of "vegetative." However, these terms are antiquated and have little or no meaning for echinoderm development, except as indicating position or direction in relation to the polar axis. The terms "apical" and "basal," and "acropetal" and "basipetal," seem equally applicable and somewhat more convenient and are used except where the others seem to be required. The terms "pole" and "antipole" have been used by some authors for the two ends of the polar axis, but "polar" and "antipolar" are less satisfactory than "apical" and "basal," and the use of "polar" to indicate one pole or the region adjoining that pole, instead of the entire physiological axis, is likely to lead to confusion.

² Von Ubisch, 1925*a, b*; 1929; 1931; 1932*a, b*; 1933; 1934; 1936*a, b*; 1938*a*.

creasing in concentration respectively basipetally and acropetally, but assumes that after isolation of parts ectodermal substance accumulates apically, entodermal substance basally, and that transplanted micromeres are not organizers, as Hörstadius maintains, by production of "entodermal" substance, but merely attract it.

These hypotheses are attempts to interpret sea-urchin development and its experimental modifications in terms of formative substances. They present interesting similarities to the attempts made by Morgan to account for reconstitution in *Tubularia* and other forms by assuming two opposed material gradients and the changes in them required by the experimental data (Morgan, 1905, and various earlier papers). It will be recalled from earlier chapters that differential dye reduction in three sea-urchin and one starfish species and differential susceptibility, as indicated by differential death and differential modification of development, suggest a somewhat different view. These data indicate presence in early stages of only one gradient, corresponding in direction to the animal gradient of Runnström; but preceding gastrulation a second gradient with the same characteristics as the primary, as regards susceptibility and dye reduction, appears in the basal or vegetal region. The high end of this gradient is distinctly higher than the high apical end of the primary gradient; and, as immigration of primary mesenchyme occurs, its cells become the most rapidly reducing cells of the blastula. A ventrodorsal gradient also becomes evident before gastrulation. The secondary gradient does not overlap the primary but simply obliterates and reverses gradient direction for a greater or less distance acropetally. Again it must be noted that these susceptibility and reduction gradients do not constitute evidence either for or against the overlapping substance gradients, though they perhaps indicate that the difference of apical and basal metabolism is not as great as Runnström and Lindahl believe; they certainly indicate that change in gradient pattern is a feature of normal development. That different substances are present at different levels of the polar axis is indicated by the pigment band of certain species (Boveri, 1901) and by dark-field observations (Runnström, 1928a), but visible granules and substances appear, in general, to be primarily results of more fundamental physiological differences along the axis. Unquestionably, a change in physiological condition in the basal region takes place preceding gastrulation, and immigration of mesenchyme and invagination of entoderm appear to be associated with this change. Both susceptibility and dye reduction

suggest rather intense activation in the basal region; a developmental activation certainly occurs.

The first two divisions of the sea-urchin egg are meridional; the third is equatorial, apical and basal cells being approximately equal. In the fourth cleavage the four apical cells divide meridionally, forming a ring of eight cells (mesomeres), the four basal cells transversely and very unequally into the four micromeres at the basal pole and four large cells (macromeres). Next the eight apical cells divide transversely and equally, forming two rings of eight cells each, designated an_1 and an_2 by Hörstadius (Fig. 145, *A*). Somewhat later the four macromeres divide meridionally, forming a ring of eight cells, then divide transversely, forming

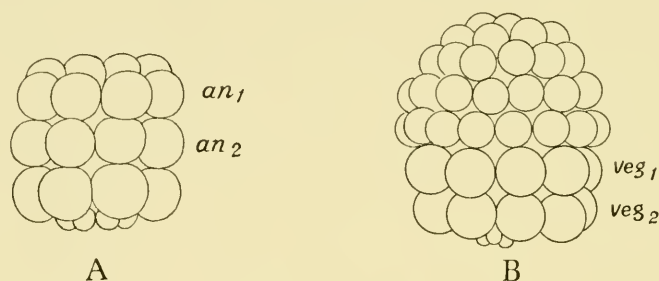


FIG. 145, *A*, *B*.—Cleavage stages of sea urchin with Hörstadius' designations of the four rings of cells used in experiment; an_1 , an_2 , apical (animal) and subapical ring derived from the four apical cells of eight-cell stage; veg_1 , veg_2 , rings derived from the four macromeres of sixteen-cell stage which are basal (vegetal) except for the micromeres.

two rings of eight cells each, veg_1 and veg_2 (Hörstadius), as indicated in Figure 145, *B*. Earlier workers disagreed as regards the portion invaginated as entoderm, but by means of vital staining of particular rings of blastomeres it seems to be demonstrated that an_1 , an_2 , and veg_1 all normally form ectoderm and that only the cells of veg_2 invaginate (von Ubisch, 1933; Hörstadius, 1935, 1936a).

With the aid of temporary exposure to calcium-free sea water Hörstadius has isolated blastomeres and groups of blastomeres as desired—even single, two, three, or four micromeres—and, since the cells adhere readily on contact, has been able to make various combinations of blastomeres or rings of blastomeres and to implant different numbers of micromeres in various relations to other cells. Staining of particular cells or groups provides a means of identification, and local staining of isolated parts serves to indicate axes, surfaces of separation, etc. In these experi-

ments the possibility exists that calcium-free sea water, isolation of micromeres or other blastomeres or groups, staining of particular cells or groups, and the manipulation necessary for transplantation may alter physiological condition of the cells concerned, either in the direction of activation or of depression; but whether such change occurs is not known. That it

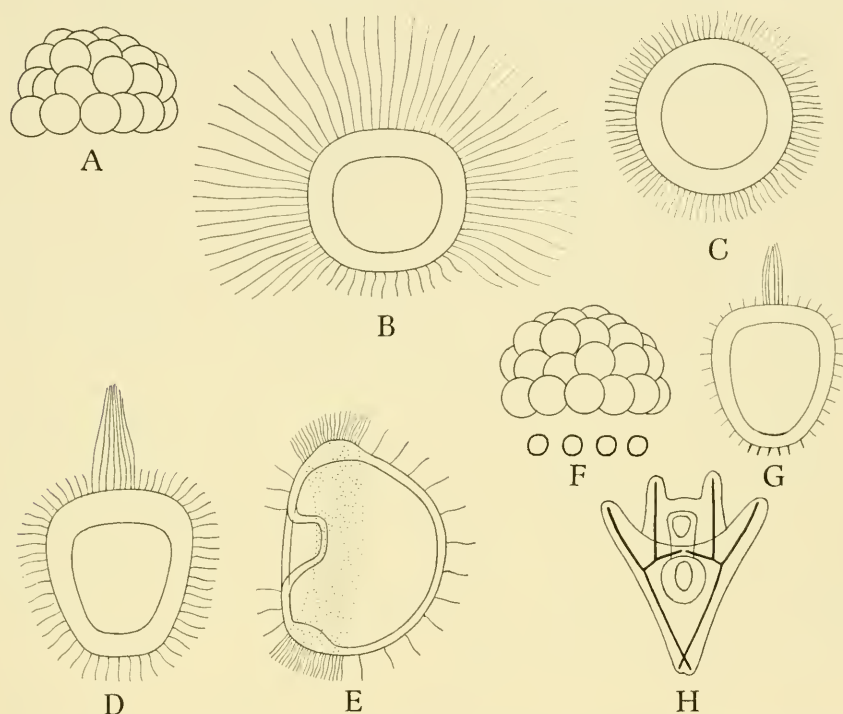


FIG. 146, A-H.—Development of isolated apical halves of *Paracentrotus*. A, isolated apical half; B, C, earlier and later stages of extreme apical partial development; D, E, earlier and later stages of less extreme apical development; F, G, H, essentially normal development following implantation of four micromeres in basal region of apical half of early blastula (after Hörstadius, 1935).

may be a factor in the variation of results of a particular experiment seems not impossible.

Apical halves, isolated in cleavage stages (Fig. 146, A), usually develop into thick-walled, blastula-like forms with extension of the apical tuft of long stiff cilia over a large part of the surface (Fig. 146, B). These are extreme apical partial forms, comparable to the apical partial forms from short pieces of *Tubularia* and *Corymorpha* (Fig. 113, A-I [p. 334]). Later

the long cilia disappear, and they become uniformly ciliated spherical forms (Fig. 146, *C*). Some apical halves, however, show less extreme apical partial development (Fig. 146, *D*), and some may finally develop ciliated band and stomodeum (Fig. 146, *E*). Apical halves differentially inhibited by lithium salts develop mesenchyme and entoderm (von Ubisch, 1925*b*, 1929) and occasionally do so in supposedly normal environment (von Ubisch, 1936*a*), though these cases may perhaps represent slight differential inhibition by some uncontrolled factor. Evidently their failure to form mesenchyme under the usual conditions is due not to lack of potency but to a scale of polar organization longer than the piece. When the scale is decreased by lithium, basal parts develop. The case is completely parallel to reconstitution of short *Tubularia* and *Corymorpha* pieces in relation to scale of organization (pp. 345-49). Animal halves with four micromeres implanted basally develop into practically normal plutei (Fig. 146, *F-H*); that is, the vegetal gradient is increased by the micromeres (Hörstadius), or the primary gradient is partly obliterated and reversed by the activation of the micromeres.

Figure 147 indicates results of implanting one, two, or four micromeres in the isolated blastomere rings. an_1 requires four micromeres for normal development; an_2 with one micromere approaches pluteus form, is normal with two micromeres, and less completely developed apically with four. Development of veg_1 is not complete in any case but becomes less complete as more micromeres are implanted, and entoderm is relatively "too large"; veg_2 , with one or more micromeres, develops exogastrulae with large entoderm and small ectoderm.

Hörstadius admits that this schematic representation is not entirely adequate, and von Ubisch (1936*a*) has called attention to the selection of particular experimental results on which it is based. Assuming, however, that it does show, in a general way, effects of difference in apicobasal level of blastomere rings and of number of micromeres present, it indicates, as regards an_1 , an_2 , and veg_1 , that the effect of implanted micromeres increases with the number implanted and with increasingly basal level of the blastomere ring. If the micromeres undergo activation sooner or later, as they apparently do preceding normal gastrulation, they probably obliterate the primary gradient and reverse gradient direction over a greater or less distance from the region of implantation; and this secondary gradient extends farther and is more effective at lower than at higher levels of the primary gradient, that is, in the more basal blastomere rings. Activation of micromeres increases the scale of basal or vegetal organiza-

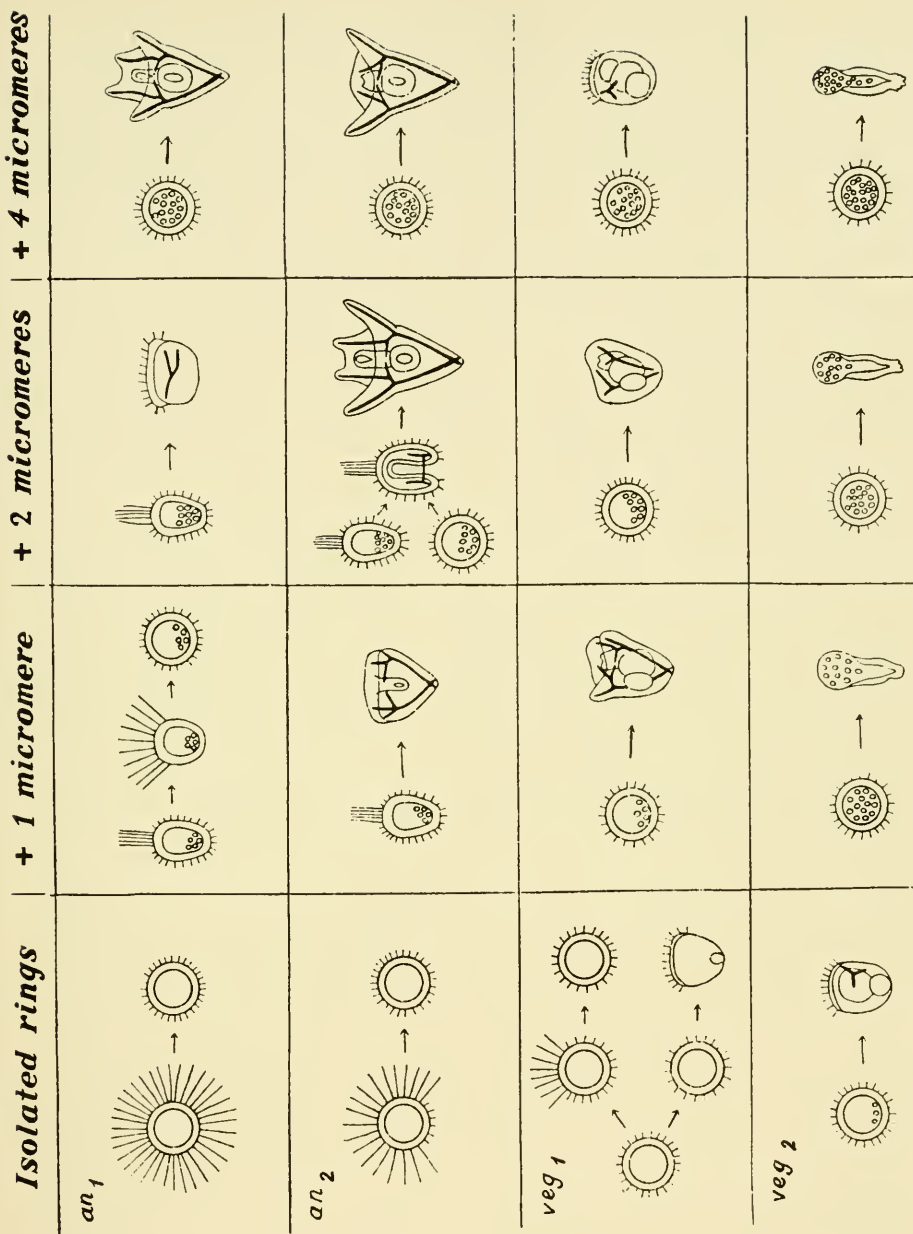


FIG. 147.—Schematic summary of results of implantation of one, two, or four micromeres basally in isolated blastomere rings, *an*₁ to *veg*₂; further explanation in text (from Hörstadius, 1935).

tion, and apical regions are correspondingly decreased. In the case of an_1 , normal development with four micromeres seems rather difficult to account for on the basis of substance gradients. An_1 represents the highest concentration of animal substance; and, since it is less than a fourth of the whole polar pattern, there can be no great difference in concentration in it. The micromeres supposedly represent the highest concentration of vegetal substance. To produce an effective vegetal gradient this substance must diffuse from the micromeres. To balance the animal gradient it must approach the concentration of normal development; but in that case we should expect no great difference in concentration of either substance, since only the two ends of the polar axis are represented. Evidently there must be mutual "suppression" between the two gradients as the vegetal substance diffuses from the micromeres, if anything like normal development is to result, or else the accumulation at the two poles of the two substances must be postulated; and these assumptions raise further questions and difficulties. Similar difficulties arise with regard to an_2 and veg_1 ; each represents only a fraction of the gradients, and the micromeres another fraction. How do whole gradients arise from these? The concept of dynamic gradients which can be altered in length, height, or slope and obliterated or reversed by quantitative metabolic differences seems to present fewer difficulties and is in line with observations on normal sea-urchin development and on reconstitution in many animals. If a substance diffuses from the micromeres, may it not be simply an activating substance rather than one producing some specific differentiation?

Effects of the micromeres with veg_2 appear anomalous, in terms of concentration gradients. The ring, veg_2 , is supposedly entirely entodermal, but there is reconstitution of ectoderm from its more apical region and, with micromeres present, exogastrulation. But, according to Hörstadius' experimental records seven of thirteen larvae from isolated veg_2 without micromeres developed exogastrulae; and of seventy-three with one or more micromeres, thirty-seven showed different degrees of exogastrulation, and thirty-six died early with little development. In normal development veg_2 does not form ectoderm at all; but when isolated, with or without micromeres, ectoderm is reconstituted. According to the substance hypothesis, it contains only a low concentration of animal substance but a high concentration of vegetal substance. How can the animal substance become effective in the presence of the high concentration of the other? The reconstitution of ectoderm in veg_2 is apparently quite similar to re-

constitution in hydroids and planarians. Isolation from higher levels of the primary gradient results in activation of the more apical parts of *veg*₂ and raising of gradient-levels there, so that ectoderm, instead of entoderm, develops; but the ectoderm does not attain full development, probably because inhibited by the basal activation and gradient, much as hydranth and planarian head are inhibited by an opposed activation gradient.

It is a question of some interest whether exogastrulation in *veg*₂, without or with micromeres, results merely from the large proportional size of entoderm or from some other condition. From Hörstadius' data it does not appear that the micromeres play any considerable part in determining exogastrulation. In this respect Figure 147 appears somewhat misleading, but it does indicate a greater degree of exogastrulation with larger entoderm and less ectoderm with increasing number of micromeres. Even if the micromeres increase concentration of vegetal substance, it is not evident how such increase determines failure of entoderm to invaginate. Since *veg*₂, with or without micromeres, is retarded in development and about half the number isolated die early, inhibition, rather than the micromeres, is probably the factor determining exogastrulation; but whether early isolation, reconstitution of ectoderm, or some other factor is the inhibitor remains uncertain. If these isolates are inhibited, exogastrulation is probably determined in the same way as with chemical agents.

After implantation of four micromeres between *an*₁ and *an*₂ of whole embryos gastrulation and entoderm formation from presumptive ectoderm occur at the region of implantation, as well as basally; and plutei with two guts and usually with extra skeletal elements result. Results are essentially similar after implantation of four micromeres between *an*₂ and *veg*₁, except that the induced entodermal invagination is nearer the original entodermal pole. Implantation of four micromeres in the apical pole of whole embryos may induce a second small entodermal invagination in that region, persisting in the apical region of the pluteus (Fig. 148, A-C), and in some cases extra skeletal elements develop. Implantation of four micromeres into the apical pole of apical half-blastulae results in a wide range of forms. In some individuals development of the apical region is more or less inhibited, but the original polarity persists; in others the implanted micromeres may give rise to extra skeleton in the apical region; in still others a small apical invagination is induced, and another occurs in the basal region of the apical half, that is, from presumptive ectoderm; and finally, in some there is a small apical invagination with complete

reversal of polarity (Fig. 148, *D-F*). As far as the experimental evidence goes, the invaginations induced by implanted micromeres may conceivably be due to an activation above the ectodermal level, such as apparently occurs normally at the time of immigration of primary mesenchyme, or to a specific effect. Incidentally, it may be noted that apical invaginations, in appearance much like those figured by Hörstadius, are present not infrequently in secondary modifications of differentially inhibited forms, even in exogastrulae. Although they appear at the extreme apical

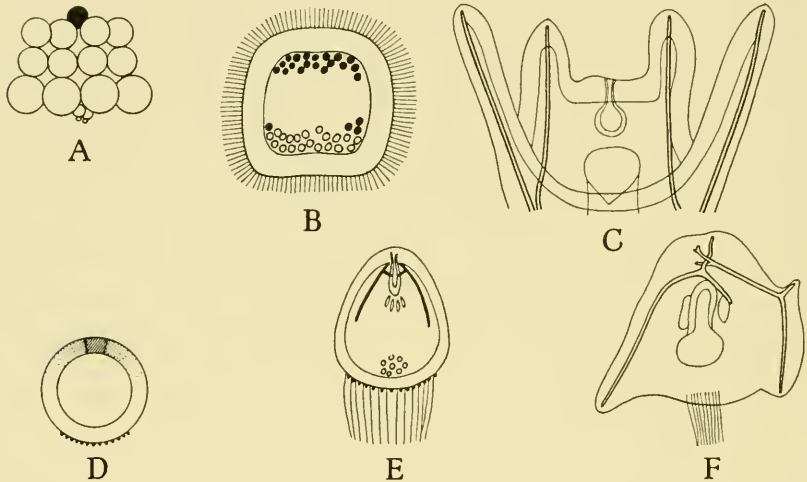


FIG. 148, *A-F*.—Implantation of four micromeres into apical pole. *A-C*, implantation in whole embryo with development of small entodermal invagination, persisting in apical region of pluteus; *D-F*, implantation of micromeres into apical region of apical half-blastula with complete reversal of polarity; stained micromeres and apical region of half indicated by shading in *D* (from Hörstadius, 1935).

pole, they have been regarded as stomodeal invaginations. To account for reconstitution of an apical region from the original basal region of the apical half-blastula (Fig. 148, *D-F*), either in terms of activity gradients or concentration gradients, requires special assumptions or hypotheses. It may conceivably represent a physiological isolation, resulting from alteration in the original apical region. When formed, the micromeres apparently represent the low end of the primary gradient; their implantation apically may interfere considerably with the primary gradient in early stages before their supposed activation and so may permit physiological isolation of the basal region and reconstitution of an apical region there. In terms of concentration gradients interpretation seems at

least equally hypothetical. Micromeres are supposed to increase the vegetal gradient. How do they determine an animal gradient opposite in direction to the original in the basal region of the half-blastula? The gradient changes required can, of course, be assumed to take place; but such assumptions are certainly no less hypothetical than those concerning physiological isolation, for physiological isolation is demonstrated in many cases.

As already noted, development of mesenchyme and entoderm from the basal regions of apical halves is brought about by certain exposures to lithium. The most basal regions of lithium-treated apical halves, marked by local staining at the time of isolation, when implanted in the basal regions of other apical halves, prevent the extreme apical partial development characteristic of isolated apical halves and induce entoderm formation, and development of essentially normal plutei may result. These implanted cells are regarded as a secondary organizer by Hörstadius (1936*b*). Cells from the apical regions of lithium-treated blastulae, similarly implanted in apical halves, perhaps have slight effect in inhibiting extension of the apical tuft; but, since they do not induce entoderm, they afford evidence that the effect of the "secondary organizer" is not due to presence of lithium in it. Hörstadius regards the action of lithium as similar to that of implanted micromeres, but the evidence from chapter vi indicates that lithium is generally inhibitory; and the production of entoderm in the lithium-treated apical halves and in those with implanted basal cells from lithium-treated apical halves is apparently quite similar to the reconstitution of more basal parts in short pieces of *Tubularia* and *Corymorpha* when scale of organization is decreased by inhibiting conditions (pp. 344-48). Activation of mesenchyme and entoderm in the lithium-treated half occurs in recovery after return to water, not as a direct effect of lithium. Heteroplastic transplantation of micromeres has shown that the inducing action and the reactions of the host ectoderm to the skeleton are not species-specific and that the skeleton in the resulting chimeras may possess characteristics of the donor species.³

In normal asteroid development there are no micromeres, nor is any mesenchyme formed preceding gastrulation; but invagination of entoderm occurs in essentially the same way as in the sea urchin, though the most intense activation of the entoderm apparently takes place after invagination (p. 137). Even in the sea urchin presence of micromeres or of primary mesenchyme, resulting from reconstitution of other parts of the

³ Von Ubisch, 1931, 1932*a,b*, 1934, 1936*b*; Hörstadius, 1936*d*; F. J. Schmidt, 1936.

embryo, is apparently not necessary for invagination. Stained cells from *veg*₂, implanted in the apical poles of whole embryos, may invaginate without reconstituting primary mesenchyme; but a part of the primary mesenchyme of the host aggregates about the invaginated cells (Hörstadius, 1935).

In view of all the evidence, not only that from the transplantation experiments but also that from differential dye reduction, differential susceptibility, and the differential modifications of development resulting from it, it may still be questioned whether induction by implanted micromeres is primarily anything more than a nonspecific effect depending on physiological condition rather than on specific differentiation; but even if the micromeres produce a specific substance, its effect may be primarily activation. In his interpretations of experimental results Hörstadius uses terms implying dynamic factors. He speaks of the "conflict" of the gradients, the "strength" or "weakness" of one or the other, the "suppression" of one by the other, the "weakening" of one, permitting the other to become stronger and gain the upper hand. In terms of primarily nonspecific gradients of rate or intensity sea-urchin development appears not as a conflict of gradients but rather as an orderly and definitely determined sequence of gradient changes which can be altered in definite ways by changes in relations of parts and in environment.

FORMAL PATTERNS OF VERTEBRATE DEVELOPMENT

Before discussing induction in vertebrate development some consideration of the formal regional maps and of the cell movements or migrations by which parts of the early embryo attain their final positions is necessary. In the study of embryonic development it soon became evident, for vertebrates as for invertebrates, that observation and description of development, necessary though it was as a first line of attack, could give us little more than a timetable of events as a basis for discussion and speculation. The observation that localization of the embryo in a particular region involved considerable changes in position and apparent migration of cell materials raised the question of the fate in development, the prospective significance, of different regions; and the theory that embryo formation resulted in large part from concrescence of two lateral halves was advanced and debated pro and con. In the attempt to determine more exactly the fates of different regions methods of local marking by puncture or other local mechanical injury, insertion of hairs, local cautery, and, later, local electrolytic injury and radiation were employed. To all these the objection has been raised that the injury or obstruction resulting from it may interfere with the cell migrations. The method of

local vital staining by application to the surface of egg or embryo of small pieces of agar impregnated with dye⁴ is open to the objection only that the dye may be, to some extent, toxic; but results obtained thus far indicate that, as the method is used, toxicity is not sufficient to constitute a serious objection to it.

This method has made it possible to map more or less accurately prospective or presumptive embryonic regions and to follow the cell migrations that bring them to their definitive positions. Such maps have, in general, little more than formal significance, since isolation and transplan-

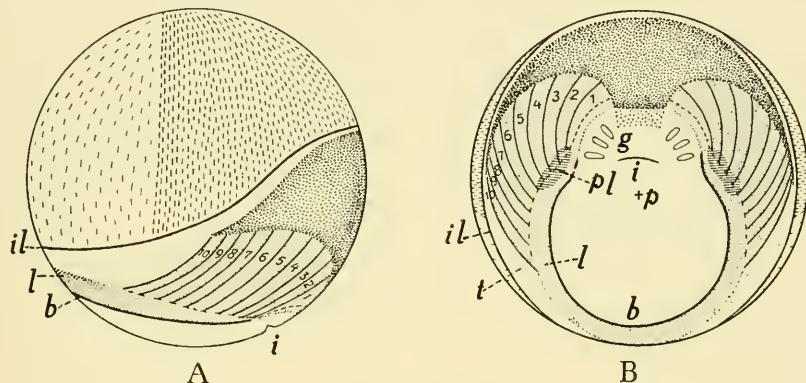


FIG. 149, *A, B*.—Map of prospective or presumptive urodele embryonic regions at beginning of gastrulation. *A*, lateral, *B*, dorsal view. Denser broken lines, neural plate; less dense broken lines, general ectoderm; coarse stippling, notochord; fine stippling, mesoderm; *i*, beginning of invagination; *b*, later blastopore; *il*, limit of invagination; *p*, basal pole; *g*, gills; *l*, lateral mesoderm; *pl*, pronephros and mesoderm of forelimb; *t*, caudal region; *1-10*, somites (after Vogt, 1929).

tation experiments have made it clear that identification of a particular embryonic area, as giving rise in normal development to a particular organ or organ system, gives no information concerning its potentialities or potencies. It shows only that, under the conditions which we call "normal," a certain region of the egg or early developmental stage becomes a certain part.⁵

The accompanying amphibian, teleost, and chick maps indicate formal regional pattern and regional migrations, as determined by local vital staining. The amphibian maps, as given by Vogt (1929), are the most complete. Figure 149 shows the urodele regional map at the beginning

⁴ Vogt, 1925; 1926*a*, *b*; 1929.

⁵ The following papers are concerned with this problem of formal pattern. *Fishes*: Kopsch, 1927; Oppenheimer, 1936*c*; Vandebroek, 1936*a*; Pasteels, 1936*a*. *Amphibia*: Vogt, 1925,

of gastrulation, viewed laterally (*A*) and from the basal pole (*B*), Figure 150, the map of the early anuran blastula, viewed laterally (*A*) and dorsally (*B*). The general similarity is evident, the chief difference between the two being that in the anuran the regions of the axial organs do not extend so far toward the apical pole as in the urodele, the greatest difference being in the neural plate. In Figure 151 directions of cell migrations (*A* and *B*) are indicated as they take place in gastrulation. With

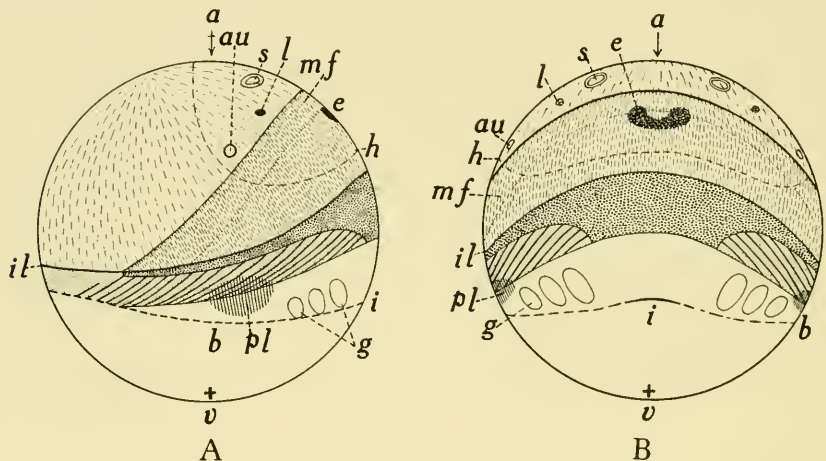


FIG. 150, *A*, *B*.—Regional map of early anuran blastula. *A*, lateral, *B*, dorsal, view; denser broken lines, neural plate; less dense broken lines, general ectoderm; coarse stippling, notochord; fine stippling, mesoderm; *a*, apical, *v*, basal, pole; *i*, beginning of invagination; *b*, later blastopore; *il*, limit of invagination; *g*, gills; *h*, posterior limit of head ectoderm; *mf*, medullary (neural) fold; *e*, field of eyes and chiasma; *s*, sucker; *l*, lens; *au*, auditory vesicle; *pl*, pronephros and mesoderm of forelimb; somites indicated by lines in mesoderm (after Vogt, 1929).

approach of gastrulation there is an increase in area and a stretching, particularly in the apicobasal direction, in cells of the apical hemisphere, apparently greatest in the dorsal region; and gastrulation begins with the “rolling in,” the invagination of cells in the median dorsal region at the boundary between the future chorda-mesoderm and yolk. This invagination extends laterally and ventrally until the blastopore becomes

1926*a*, *b*, 1929; Pasteels, 1932. *Birds*: Assheton, 1896; Peebles, 1898, 1904; Wetzels, 1925*a*, *b*, 1929, 1931, 1936; Kopsch, 1926*a*, *b*, 1934*a*, *b*; Gräper, 1929; Pasteels, 1926*b*, 1937*a*; Jacobson, 1938. For citation and discussion of the literature, including earlier papers concerning concrescence, gastrulation, and vertebrate developmental pattern in general, see the above authors, particularly Vogt, 1929; Wetzels, 1931; Pasteels, 1937*a*; also Holmdahl, 1925, 1926, 1933, 1935; and textbooks of vertebrate embryology.

circular, gradually decreases in diameter, and overgrows the yolk. The invaginated cells migrate anteriorly beneath the ectoderm and give rise to notochord and mesoderm, and continued increase in area of the superficial cells brings more material to the blastopore lip and so to invagination. Isolation and transplantation experiments have brought to light various facts of interest concerning these regional changes in position of cellular material. Vogt (1923) maintains that they are not the summation of activities of single cells but essentially regional amoeboid movements in

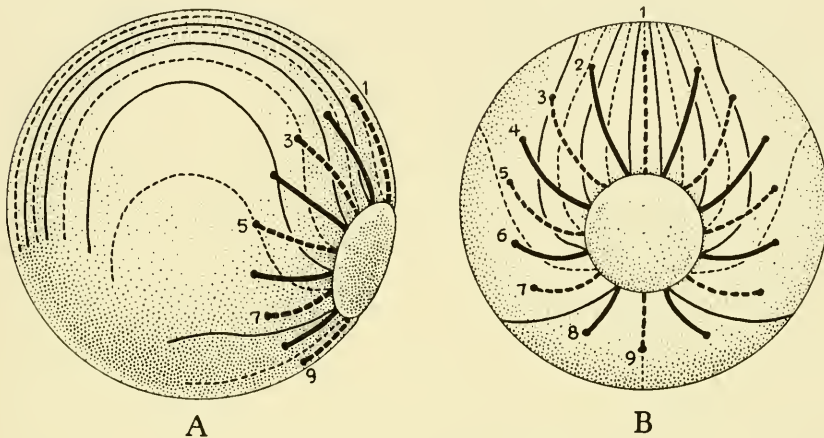


FIG. 151. *A, B*.—Directions of cell migrations in amphibian gastrulation. *A*, lateral, *B*, dorsal view; alternation of broken and unbroken lines merely for ease in following directions (from Vogt, 1929).

which the individual cells are passive. Pieces of the future blastopore lip transplanted to other regions undergo the same “stretching” and invagination as in normal environment, even when abnormally oriented with respect to the region in which they are implanted, and different regions of the blastopore lip stretch and invaginate independently. When invagination is prevented in transplanted pieces or united halves of embryos, hornlike or irregular outgrowths arise. Also, stretching and invagination may occur independently of each other. According to Holtfreter, however, the ability to stretch is intrinsic in individual cells; isolated blastula cells undergo the change of shape in the same way as larger cell groups.⁶

Thus far no physiological interpretation of these changes in shape and migrations of cells has been offered, but it is perhaps of interest to note

⁶ O. Mangold, 1920; Vogt, 1922*b*; Spemann und H. Mangold, 1924; Spemann, 1931, 1936, pp. 63–71, 1938, pp. 101–10; Holtfreter, 1939*b*.

that they apparently occur first and are greatest in those regions of the embryo which show the highest differential susceptibility, the most rapid dye reduction, and perhaps the highest respiration (pp. 151-58), and the stretching is greatest in the longitudinal direction. These relations suggest that they are related to the gradient pattern of the stages concerned. It appears difficult to account for stretching in a particular direction unless there is a differential of some kind in this direction, affecting individual cells. The isolated and transplanted pieces, or even individual cells, represent a part of this differential and behave accordingly. As Vogt and Spemann have pointed out, the movements constitute an orderly, definite series of events which determine a definite result. If the cells were all alike and there were no regional directive factor, this would be impossible. Such evidence as is available indicates that the cells initiating invagination in the intact embryo represent the highest levels of the gradient system of the dorsal lip region. In the absence of this region in transplanted pieces the highest gradient-level present apparently initiates invagination. Increase in surface area of cells occurs in early embryonic development of many other forms and apparently begins in the high regions of the gradient pattern present. For example, the ectoderm of the echinoderm blastula and gastrula undergoes increase in surface area, beginning apically and progressing basipetally, and the developmental activation of the entoderm is followed by increase in its surface area. The relations of these changes to gradient pattern are evident; and when the gradient pattern is experimentally altered or obliterated, they are also altered.

In the amphibian the regional differences in these cell activities appear to be quantitative rather than limited to particular regions, but they may be regarded as representing a certain stage of development from the primary gradient pattern, this stage being attained first and in greatest degree by cells of the higher gradient-levels. Whether, or to what extent, the cells concerned have become specifically different from others which attain the condition later or not at all remains to be determined. The autonomous invagination of pieces of the blastopore lip suggests a "determination" of some sort. The factors determining invagination are unknown, but, in general, gastrulation appears to represent a certain stage in the progressive complication of the earlier gradient pattern. This seems particularly clear in echinoderm development.

The regional map of the teleost blastoderm, as determined by Pasteels (1936a), is shown in Figure 152, A; and the directions of cell migrations

in relation to gastrulation, in Figure 152, *B*. Maps by Oppenheimer (1936*d*) are very similar. Regional maps of the chick blastoderm by Gräper (1929), Wetzel (1929), Waddington (1932), Pasteels (1936*b*), and Jacobson (1938) are, in general, much alike but differ in certain minor points which cannot be considered here. Maps of four stages by Pasteels are shown in Figure 153, *A-D*; and in Figure 154, *A-D*, directions of cell migrations are indicated. Superficial migrations in formation of the primi-

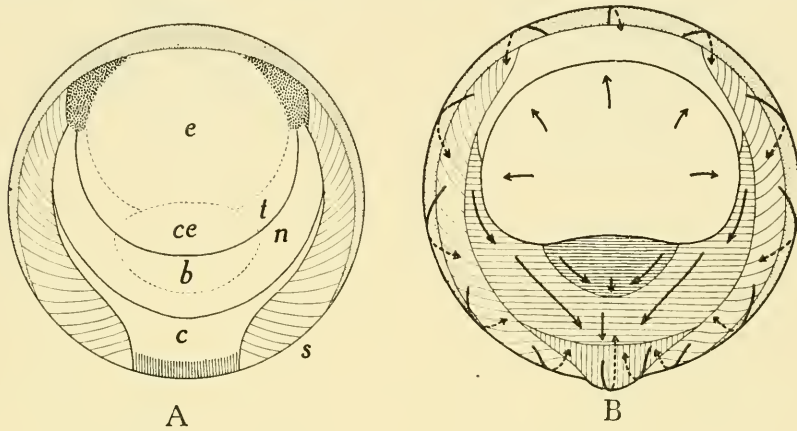


FIG. 152, *A, B*.—*A*, map of prospective embryonic regions of teleost blastoderm just preceding gastrulation; *B*, directions of cell migrations in gastrulation. Vertical lines, prechordal plate; lightly stippled, lateral and ventral mesoderm; heavily stippled, caudal unanalyzed regions; *b*, brain; *t*, trunk ectoderm; *c*, notochord; *ce*, cephalic ectoderm; *e*, extraembryonic ectoderm; *n*, postcephalic nervous system; *s*, somites (from Pasteels, 1936*a*).

tive streak, as determined by Wetzel, are shown in Figure 155, *A-C*. Jacobson believes that the longitudinal migration recorded by these authors does not take place. In spite of the general similarity of results, there has been disagreement as regards significance of the primitive streak. The more generally accepted view is that the streak represents a part of the gastrulation process in which mesoderm is invaginated, entoderm being invaginated earlier from the posterior border of the blastoderm (e.g., Patterson, 1909). Wetzel, however, maintains that the streak has nothing to do with gastrulation.

It has become evident that the formal regional pattern and the migrations concerned in embryo formation are, in general, similar in all vertebrate groups. Even mammalian development, although it exhibits certain features associated with intrauterine environment, evidently does not dif-

fer fundamentally in general pattern of embryo formation from that of other vertebrates. Moreover, pattern of embryo formation in *Amphioxus* and ascidians shows certain general resemblances to that of vertebrates.

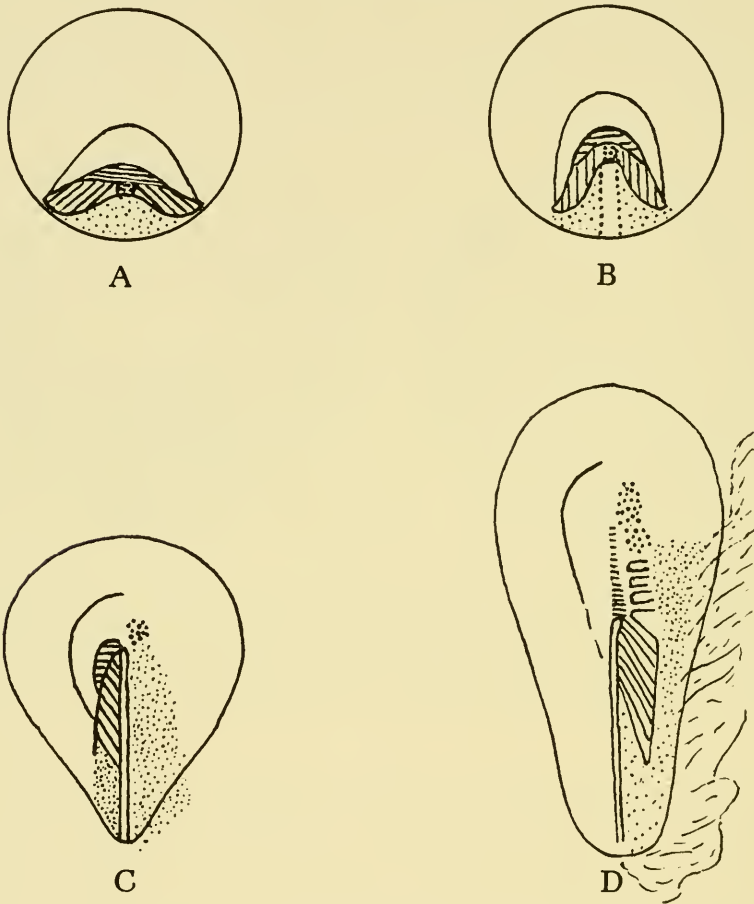


FIG. 153, *A-D*.—Regional map of chick embryo at four stages; dotted area, lateroventral mesoblast; oblique or vertical lines, somite region; coarse stippling, cephalic mesoblast; horizontal lines, notochord; neural region indicated by line marking its anterolateral boundary, *A*, early blastoderm; *B*, early primitive streak, parallel dotted lines indicating streak; *C*, *D*, later stages, showing superficial map on left side, map of invaginated parts on right (after Pasteels, 1936b).

As already pointed out, the regional patterns indicated by the maps give no information concerning determination or differentiation of the regions distinguished. At best they are maps of future embryonic parts.

The regions mapped are not, in general, coextensive with embryonic fields. Usually they are more limited than the fields, that is, they show the regions which will actually develop into certain organs, not the regions in which development of those organs is possible. Physiologically, such a map may represent nothing more than a quantitative gradient pattern,

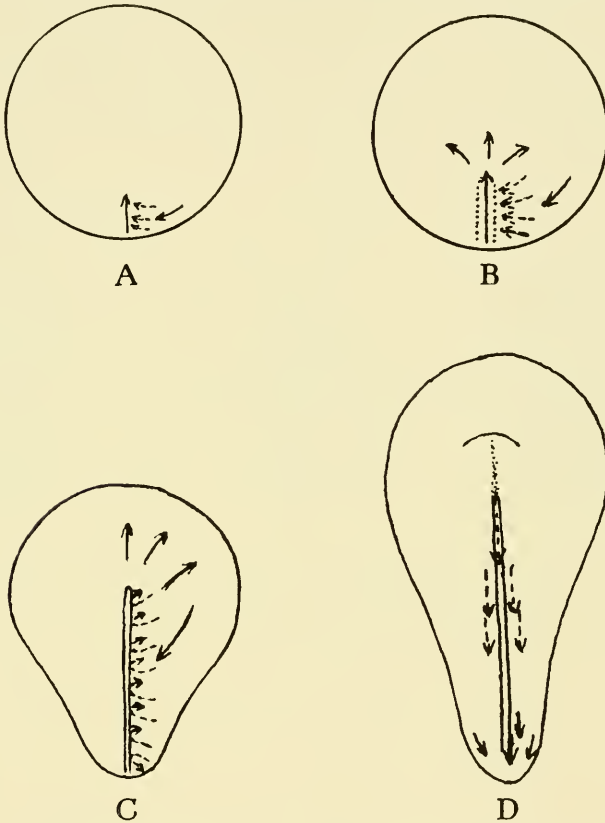


FIG. 154, A-D.—Cell migrations in chick embryo at four stages, according to Pasteels; arrows drawn in unbroken line indicate movements involving both cell layers; those in broken line, movements of surface layer only (after Pasteels, 1936b).

the developmental fate of the regions being determined by their position in the gradient system. In other words, the regional maps of early stages do not represent actual patterns but are projections of the patterns of later stages back onto a stage in which the actual pattern is simpler and more general in character. It is not necessary to think of the various parts of the embryo as spread out on the blastula or blastoderm, as the maps

seem to indicate. The parts are not necessarily present in these stages, but the future pattern is projected on a present pattern, from which it originates. The cell migrations constitute the basis for projection. That dynamic factors of some sort play a part in determining and directing these movements seems probable, and the possibility that electric-potential gradients may be concerned in determining the differential in the cells suggests itself. An anteroposterior potential gradient is reported in *Amblystoma* and chick embryos, with increase in potential difference with progress of development and demonstrable without direct contact with the embryo. Also, the dorsal lip region of *Amblystoma* is electronegative

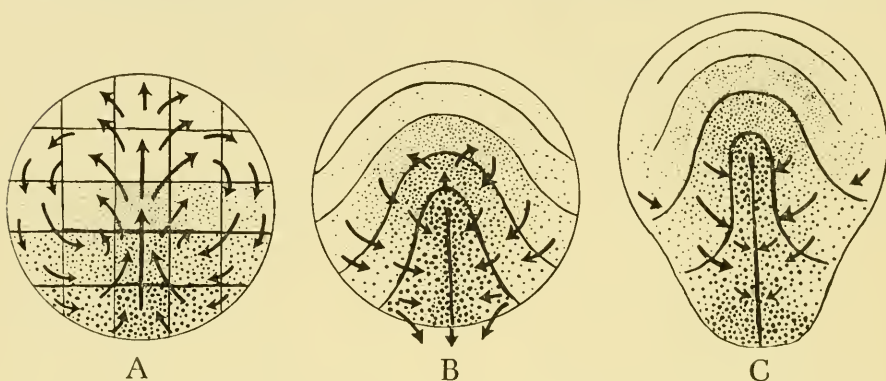


FIG. 155, A-C.—Cell migrations in chick embryo, according to Wetzel; change in position of embryonic regions indicated by gradations of stippling. A, directions in which migrations will occur in formation of primitive streak; B, C, later streak stages (from Wetzel, 1929).

to the apical pole. The developing embryo apparently gives rise to a steady-state electrodynamic field with definite axiate and regional intensities (Burr and Hovland, 1937*a, b*).

The recent studies of cell migrations by means of local staining have finally shown that the much debated concrescence theory of vertebrate development, that all except the most anterior parts are formed by union of two originally separated lateral halves, is not entirely in accord with the facts. The axial organs are primarily median, but there is concrescence or convergence of certain lateral areas toward the median plane.⁷

NEURAL INDUCTION IN AMPHIBIAN DEVELOPMENT

The work on induction and so-called "organizers" in amphibian development has attracted much attention and has become widely known

⁷ See the discussion by Vogt, 1929, pp. 668-78.

through its consideration in handbooks, textbooks, general reviews, and addresses.⁸

The induction which has aroused most interest and received most attention, supposedly the earliest induction in amphibian development, is the induction of the neural plate by the dorsal region which invaginates, underlies the ectoderm which becomes neural plate, and itself becomes chorda-mesoderm. Stages of invagination of chorda-mesoderm and ectoderm and the neurula, with neural plate formed after invagination, are shown in Figure 156. As is now well known from the work of many investigators, a piece of the presumptive chorda-mesoderm region (Figs. 149, 150), transplanted before its invagination to a region not normally involved in neural-plate formation, or implanted in the blastocoel of another embryo, can induce a new neural plate in ectoderm of the host which would normally form only epidermis (Figs. 157, 158). The secondary embryo thus induced was at first regarded as a product of the implant rather than an induction (Spemann, 1918), but heteroplastic transplantations from the dorsal lip of the unpigmented *Triton cristatus* to the pigmented *T. taeniatus* made it evident that the neural plate developing in relation to the implant was wholly, or almost wholly, derived from host tissue, while the underlying chorda-mesoderm was derived from the implant.⁹

Transplants of presumptive ectoderm had no such inducing effect but were incorporated and developed according to their position in the host, although preserving their pigmentary species-characteristics. The forms resulting from these heteroplastic transplantations are chimeras, consisting of tissue of two species. These experiments led Spemann to regard the inducing region as an "organizer" or "organization center." Only some of the more important points in the further investigation of various aspects of this induction by Spemann and many others are considered here.

⁸ See the following books: Morgan, 1927, a brief account; fuller consideration with extensive bibliographies in Schleip, 1929; Huxley and De Beer, 1934; Daley, 1935, on chordate egg organization in general, with bibliography; Spemann, 1936, 1938, lectures concerned with the problem of induction in embryonic development, with bibliography; Weiss, 1939, a general textbook of experimental embryology; also the most recent book on the subject, Waddington, 1940, *Organizers and Genes*; a consideration, from the viewpoint of the Cambridge group, of evocators and organizers in vertebrate, and chiefly in amphibian development, with discussions of competence, individuation, and organization in general, and an attempt to bring genes into the picture, but without anything new in the way of synthesis or further light on the problems concerned. Numerous reviews of the subject have also appeared in *Ergebnisse, Jahresberichte*, and review journals, notably those of Mangold, 1928a, 1929a, 1931a, with bibliographies. See also De Beer, 1927; Gilchrist, 1929c; Weiss, 1935.

⁹ Spemann, 1921; Spemann und H. Mangold, 1924. See also Marx, 1925; Geinitz, 1925a.

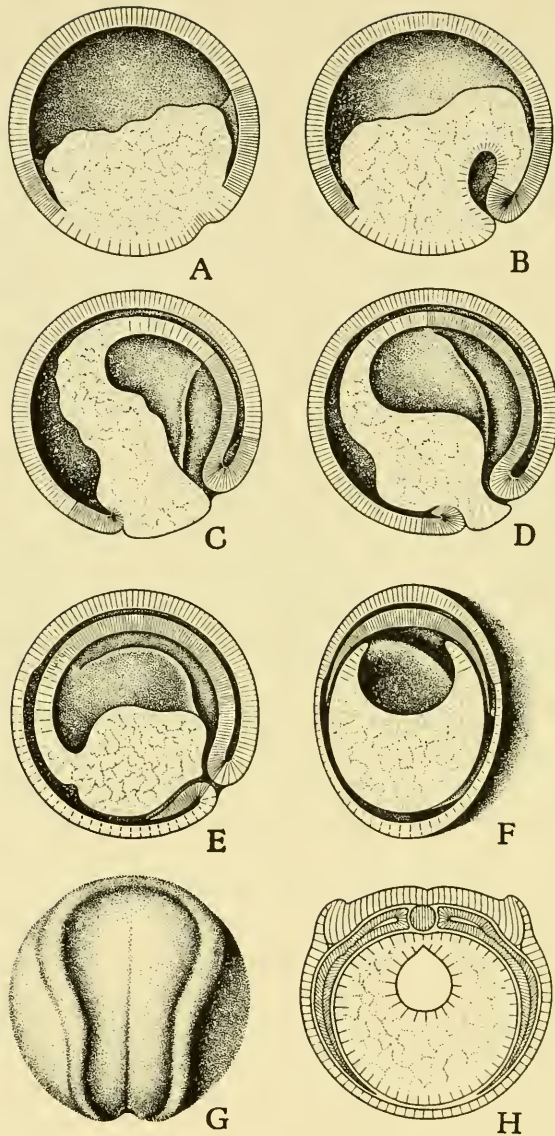


FIG. 156, A-H.—Amphibian gastrulation and neural plate formation; diagrammatic; chorda-mesoderm indicated by deeper, ectoderm by lighter line-shading, entoderm by cell boundaries. A-E, median sections; F, same stage as E in transverse section; G, H, neurula in dorsal view and in transverse section (from Spemann, 1936, *Experimentelle Beiträge zu einer Theorie der Entwicklung*. Springer, after chart by V. Hamburger and B. Meyer; also Spemann, 1938).

The earlier experiments were on urodeles, chiefly *Triton*; but work with anura and heteroplastic and xenoplastic transplantations between different urodele species, between anuran species, and even between urodeles and anura have shown beyond question that the presumptive chorda-mesoderm acts as inductor in amphibians generally and that its action is not species-, genus-, or even order-specific; but tissues and even embry-

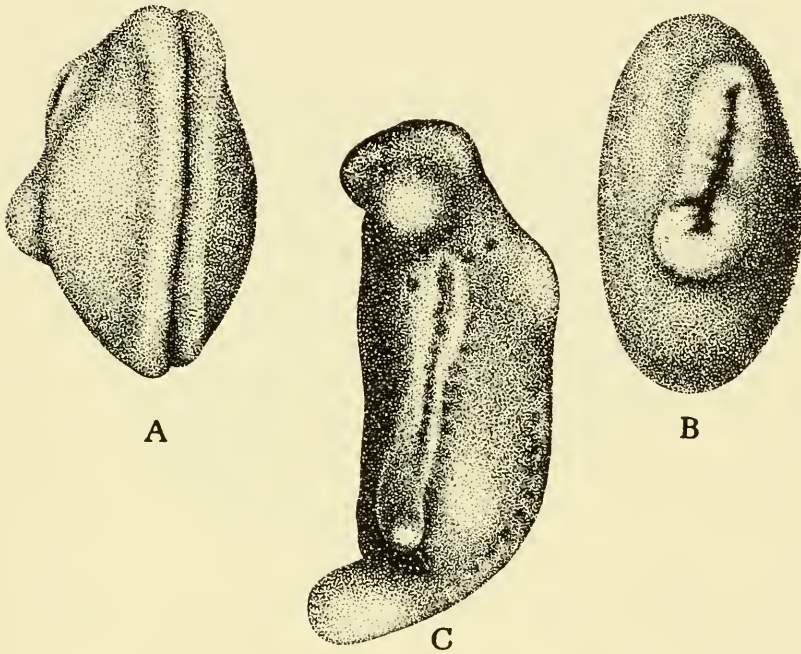


FIG. 157, A-C.—Induction of a secondary neural plate and embryonic axis in ectoderm by implantation of dorsal lip material. A, B, two views of *Triton taeniatus* neurula with secondary neural plate induced by heteroplastic implant from dorsal lip of *T. cristatus*; C, later stage of same embryo with secondary embryo on left side (from Spemann und H. Mangold, 1924).

onic extracts of some forms are toxic for some other species.¹⁰ Since action of the living dorsal inductor, is, to such an extent, independent of species, the question at once arises as to the nature of its action, whether material or dynamic. This question has been raised and discussed by

¹⁰ Geinitz, 1925a; Bytinski-Salz, 1929a, b, c; H. Mangold, 1929b; O. Mangold, 1929b; Schotté, 1930; Raven, 1931, 1933b; G. A. Schmidt, 1933, 1936a, b; Holtfreter, 1935a, b, 1936. See also Twitty, 1937, "Experiments on the phenomenon of paralysis produced by a toxin occurring in *Triturus* embryos," *Jour. Exp. Zool.*, 76; Horsburgh, Tatum, and Hall, 1940, "Chemical properties and physiological actions of *Triturus* embryonic toxin," *Jour. Pharmacol. Exp. Therapeutics*, 68.

Vogt, Spemann, Mangold, and more recently by others. But quite apart from this question, the lack of species-specificity suggests two possibilities in this induction: production by different species and even by urodeles and anura of the same, or closely similar, substances, with essentially the same specific effects; or an activating action dependent on the high physiological level of the inductor and nonspecific. In other words, does the inductor determine wholly or in part the specific character of the neural

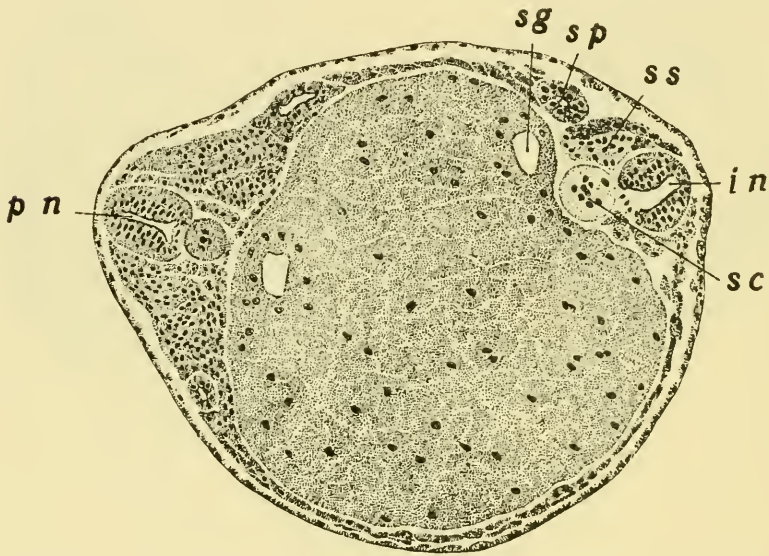


FIG. 158.—Transverse section through middle region of embryo shown in Fig. 157, *D*. Primary embryo at left, secondary induced embryo at right of figure: *pn*, primary neural tube; *in*, induced neural tube; *sc*, secondary notochord; *ss*, secondary somite; *sp*, secondary pronephric duct; *sg*, secondary gut. The implant, shown in lighter shading, forms the secondary notochord and part of the right somite (after Spemann und H. Mangold, 1924).

plate, or does it bring the ectoderm of a particular developmental stage to a physiological level at which development of a neural plate takes place? Consideration of further data must precede discussion of this question.

Both capacity of presumptive chorda-mesoderm to induce and of ectoderm to react change in the course of development. Exactly when inductive capacity appears in the living embryonic inductor is difficult to determine because pieces from earlier pregastrula stages implanted in other embryos continue to develop and may induce, and ectoderm is developing at the same time. The reactivity of ectoderm gradually decreases in later gastrula stages (Holtfreter, 1938*a*).

THE LATERAL DIFFERENTIAL IN INDUCING CAPACITY

Inducing capacity of the dorsal lip region before invagination decreases laterally from the median region, and the same is true of the inductor after invagination; the somite region is less effective than the chorda primordium, and the lateral mesoderm still less effective.¹¹ The lateral decrease in ability to induce neural plate is steeper in anura than in urodeles; lateral and lateroventral parts induce tail, not neural plate (G. A. Schmidt, 1936*a, b*; Schechtman, 1938). There is, in short, a gradient in inducing power, decreasing from the median region laterally; susceptibility of the dorsal lip region shows an essentially similar gradient (p. 152). In this connection an experiment of Waddington's (1936*a*) is interesting. He finds that transplanted, newly formed lateral-plate mesoderm is able to induce an extra neural plate from presumptive ectoderm. Median dorsal lip region, substituted for presumptive lateral plate, also induces an extra neural plate; but lateral mesoderm does not induce a neural plate in normal development. These results suggest a relation of dominance and subordination between higher and lower levels of the mediolateral gradient of the presumptive chorda-mesoderm, the high median level being dominant. When isolated from this dominance, the lateral region is able to induce, perhaps because of some degree of activation following isolation, as in invertebrate reconstitution. Lateral symmetry or asymmetry of the inductor does not determine symmetry or asymmetry of the induced plate. A right or left half of the dorsal lip region induces a whole neural plate, not a right or left half; and median or lateral pieces less than half also induce a whole plate, though not always a completely symmetrical one. The implanted lateral half of the inductor from an early gastrula reconstitutes to a bilaterally symmetrical system with median chorda. Halves from more advanced stages show less reconstitution, the notochord developing more or less at one side, and the other side being formed by more or less appropriation (induction) of host tissue. The later the stage of the implant, the more asymmetrical is the induced neural plate.¹² These facts again suggest that the mediolateral differences are primarily quantitative gradient differences but become increasingly specific with progress of development.

¹¹ Bautzmann, 1926, 1928, 1929*b*, 1933. See also Ruud, 1925; Mangold und Seidel, 1927; and various other papers include evidence on this point.

¹² Spemann, 1918; Weber, 1928; B. Mayer, 1935.

THE LONGITUDINAL DIFFERENTIAL IN INDUCTOR
AND IN REACTING ECTODERM

The region of the dorsal inductor lying nearest the level at which blastopore formation begins, and invaginating first, migrates anteriorly after invagination and finally comes to underlie the future head region, while parts which invaginate later underlie successively more posterior regions. In the attempt to determine whether a longitudinal differential or specificity in inducing capacity exists, pieces of the dorsal lip at different stages of gastrulation have been implanted. Pieces from the earliest stages of gastrulation implanted at the level of the future head induce neural plates with well-developed head region, but when implanted at trunk-levels also induce well-developed heads. Pieces from the dorsal lip of later stages implanted at head-levels may also induce neural plates with anterior head region approaching normal; but when implanted at more posterior levels, the anterior region of the induced neural plate is deficient or absent. Pieces of the dorsal inductor invaginating last and lying farthest posteriorly after invagination may induce tail.¹³ These results have led to the designation of different levels of the inductor as "head-inductor," "trunk-inductor," and "tail-inductor." Essentially similar results have been obtained with implants of different regions of the chorda primordium alone (Bautzmann, 1928, 1929*b*). However, as Spemann has pointed out, the longitudinal differences in results depend not only on regional difference in the inductor but also on a difference in the reacting ectoderm, for "trunk-inductor" implanted at head-level induces head more or less completely and is completely trunk-inductor only at more posterior ectodermal levels.

Before invagination the physiological gradient in the inductor decreases anteriorly from the region invaginating first, and in the ectoderm of the presumptive neural plate and in other ectoderm there is a gradient differential decreasing from the region about the apical pole (pp. 151-58). After invagination the two gradients coincide in direction and more or less closely in extent, in the region developing as neural plate, the one underlying the other. When the high gradient-level of the inductor is implanted at the high ectodermal level, a well-developed head results; but the high inductor level is also able to induce more or less complete head development at lower levels of the ectodermal gradient, and a lower inductor level may induce head when implanted at the high ectodermal

¹³ Spemann, 1931, 1936, pp. 167-74, 1938, chap. xiii; Holtfreter, 1933*e*, 1936; Bytinski-Salz, 1931; O. Mangold, 1932*a*; Lehmann, 1932; Schechtman, 1938.

level. Are the longitudinal differences in induction and reactivity primarily anything more than these gradient differences?

The question of longitudinal regional specificity of the inductor has been much discussed. The chorda-mesoderm is regarded as a mosaic of specific inductors by Holtfreter, and Lehmann believes he has obtained evidence of regional specificity by the use of LiCl.¹⁴ In fact, Lehmann holds that action of chemical agents on embryonic developmental stages is, in general, regionally specific. As regards his experiments, however, it must be noted that the possibilities of differential recovery and of alteration of nonspecific differential susceptibilities in the course of development by alteration of activity are completely ignored.

This hypothesis of longitudinal regional specificity in the inductor presents difficulties. Neither inductor nor presumptive neural plate gives any evidence of specific regional differences in earlier stages, though this does not prove their absence. Moreover, different levels of the central nervous system all differentiate into cells and fibers; the specificity of the different levels appears to be in the relations of these rather than a chemical specificity, but how the inductor can determine these relations does not appear. Head-inductor and trunk-inductor induce trunk or head according to level of implantation, and head is not present or absent but shows various degrees of gradation between complete development and absence. This does not suggest specificity of induction. Head and trunk, of course, become widely different in later stages, and various other inductions occur in their development; but that the neural inductor is anything more than a factor in determining relative physiological levels in the reacting ectoderm remains to be proved. The regional differences in the inductor are regarded as quantitative by Dalcq and Pasteels.¹⁵

The inductor region may be specifically different from other regions of the egg cytoplasm at the beginning of development; but, as will appear, a great number of tissues, living and dead, tissue extracts, and chemical substances are also neural inductors; consequently, any close relation between whatever specificity may be present in the natural inductor and its inducing capacity does not appear probable. According to Holtfreter (1939a), there is complete lack of specificity in aggregations of isolated amphibian cleavage cells, but with isolation at later stages pure ectoderm and pure entoderm may aggregate.

¹⁴ Holtfreter, 1938, and various earlier papers; Lehmann, 1936a, b, 1937a, c, 1938b, c.

¹⁵ Dalcq, 1935, 1938a; Dalcq et Pasteels, 1938; Pasteels, 1938. See also Pasteels, 1938, "Recherches sur les facteurs initiaux de la morphogénèse chez les Amphibiens Anoures. I," *Arch. Biol.*, 49.

IS INDUCTION NECESSARY FOR NEURAL-PLATE FORMATION?

From his experiments Goerttler (1925, 1926, 1927) concluded that orientation of an inductor implant may be favorable or unfavorable, according to its relation to the regional cell migrations; consequently, he regarded these migrations as factors in determination of the neural plate and the presence of underlying chorda-mesoderm as not absolutely necessary. Holtfreter (1933*a, b*) found orientation without effect and concluded that induction, not the migrations, is the essential factor in development of the neural plate. However, Goerttler has described and figured cases in which more or less development of neural plate and neural tube occurred when the whole dorsal lip region had been removed before its invagination. According to Lehmann (1926, 1928, 1929), there is a quantitative relation between degree of defect in chorda-mesoderm, resulting from experimental removal of part of the dorsal lip region before invagination, and degree of development of the overlying region of the neural plate; but this relation differs at different levels. Anteriorly only the most extreme defects in the inductor determine defects in neural plate, but farther posterior the neural plate is less independent. Lehmann concludes that there is labile determination of the neural plate at the beginning of gastrulation, decreasing from the anterior region posteriorly, and therefore independent of underlying chorda-mesoderm.

Isolation of parts of the embryo before, or at beginning of, gastrulation give different results. Apical regions isolated in water never develop neural plate, nor do two such regions unite (Spemann, 1936, p. 111). Cultured in balanced salt solutions, entoderm and mesoderm show a high degree of independent differentiation (W. Erdmann, 1931; Holtfreter, 1931*a, b*), and Erdmann found that presumptive neural plate similarly cultured develops into neural tissue, both in urodeles and anura; but Holtfreter maintained that it always forms epidermis, with at best only minute traces of neural tissue. However, he did obtain neural and other tissues from a group of four cells from the lower part of the apical hemisphere, isolated by destroying the other cells and developing in salt solution, surrounded by the debris of the killed cells. Erdmann and Holtfreter agree that presumptive neural plate cultured in explant does not give rise to chorda-mesoderm. Material of the dorsal lip shows a considerable capacity for reconstitution; an explanted half may give rise to a bilaterally symmetrical complex, consisting of a chordal strand with muscle and, in the anterior region, neural tissue and epidermis, that is, ectodermal derivatives from presumptive mesoderm (Holtfreter, 1933*e*).

Explanted presumptive entoderm develops as entoderm and may attain a high degree of differentiation.

Various earlier attempts to culture isolated parts of amphibian blastulae and gastrulae in organic media proved unsuccessful, but it was discovered that pieces introduced into the coelom of an older larva would live and develop. Development of these coelomic cultures differs in certain respects from that in salt solutions, as regards presumptive epidermis and neural plate (Holtfreter, 1929, 1931*a*). Presumptive neural plate may develop either into pure nervous tissue or into epidermis with practically equal frequency; and presumptive epidermis, into epidermis with differentiation of gland, ciliated, and pigment cells or into nervous tissue. Implantation in the eye cavity of older individuals after removal of the eye again gives different results; chorda-mesoderm may develop from almost all regions of the early gastrula of *Triton* (Kusche, 1929), even from the region of the apical pole in *Amblystoma* (Bautzmann, 1929*a*).

Developing eggs of the urodele *Triturus*, subjected to a lateral temperature gradient, may develop on the warm side thickenings resembling neural tissue in structure from regions of presumptive epidermis not underlain by chorda-mesoderm and typical neural folds from regions with underlying mesoderm (Gilchrist, 1928, 1929*a*, 1933). Other experiments with localized high temperatures have not produced clearly identifiable neural tissue (Castelnuovo, 1932; Margen and Schechtman, 1938). Inhibition of one-half of the embryo by low temperature or by lack of oxygen results in asymmetry and alteration in developmental fate of various parts. It shows, further, that different regions of the neural plate may develop independently of each other and that the anterior head region may develop when gastrulation is almost completely suppressed (Vogt, 1927; 1928*a, b*).

Naked embryos (axolotl) developing from early blastula stage in a modified Ringer solution undergo exogastrulation, the mesentoderm evaginating instead of invaginating and in the complete exogastrula never underlying any part of the ectoderm (Holtfreter, 1933*d, e*). Directions of cell migrations in exogastrulation are indicated in Figure 159, *A, B*; the differentiation, in *C*. Ectoderm of these exogastrulae forms an irregular cell mass, not a definite layer, and shows no traces of neural plate or neural tissue and no evidence of any definite developmental pattern. It may become ciliated, but the ciliary beat is not definite in direction, as in the normal animal. The evaginated mesentoderm, on the other hand, undergoes a high degree of differentiation into notochord, muscles, kidney tubules, and gut and may approach the normal embryo in general form,

but is, of course, inside out (Fig. 159, C; Fig. 160). All gradations between complete exogastrulation and normal embryos occur. In partial exogastrulae evagination is followed by more or less invagination; and, if this is sufficient to bring chorda-mesoderm beneath ectoderm, neural tissue may develop, varying in form and size with form and size of invaginated chorda-mesoderm and differentiating into the more posterior parts of the neural tube, or only the caudal portion when only a small part, representing the posterior portion of the chorda-mesoderm, invaginates. Pieces of

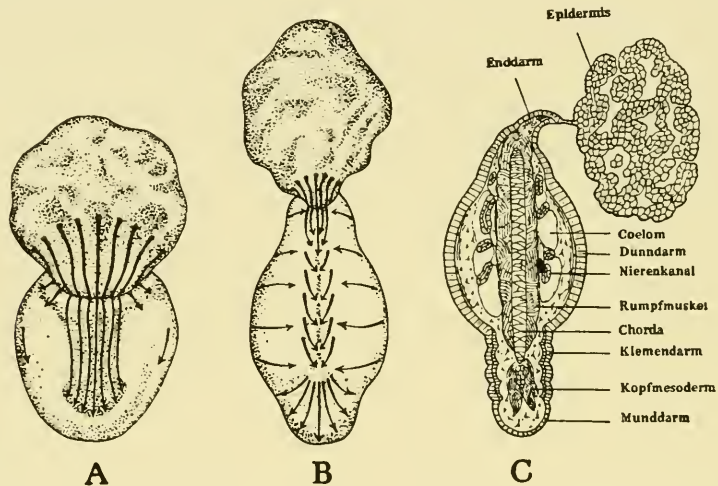


FIG. 159, A-C.—Exogastrulation in axolotl. A, B, directions of regional migrations; C, diagrammatic longitudinal section, indicating differentiation of mesentoderm and lack of differentiation of ectoderm. Parts indicated: coelom, intestine (*Dünndarm*), pronephric tubule (*Nierenkanal*), trunk muscles (*Rumpfmuskel*), notochord (*Chorda*), pharynx (*Kiemen-darm*), head mesoderm (*Kopfmesoderm*), oral entoderm (*Munddarm*), rectum (*Enddarm*), epidermis (from Holtfreter, 1933e, *Biol. Zbl.*, 53, H. 7, 8, Thieme).

ectoderm from early gastrulae, transplanted to the surface of the evaginated chorda-mesoderm, differentiate according to the region of the inductor on which they lie. On the head inductor they may develop into brain, with eyes, olfactory pits, and ear vesicles; on the trunk inductor they develop spinal cord; and at levels still farther posterior a tail may develop, with growth of chorda-mesoderm into the ectoderm and induction of a neural tube. These exogastrulae are regarded by Holtfreter as providing complete proof that there is no determination in the ectoderm before invagination of the chorda-mesoderm and, consequently, that its development is entirely the result of induction in the normal individual. This conclusion ignores, or regards as erroneous, evidence from earlier

work which is not in accord with it, as Huxley and De Beer (1934, p. 493) have pointed out; and there are other points to be considered. The fact that the presumptive neural-plate region of the exogastrulae undergoes more stretching than presumptive epidermis indicates a difference of some sort, though it may be a nonspecific gradient difference. Moreover, the Ringer solutions that bring about exogastrulation may inhibit development to some extent; various Ringer modifications inhibit planarian reconstitution. If this is the case, the exogastrulae do not provide the final proof of complete dependence of neural development on induction.

The results with presumptive epidermis and neural plate cultured in salt solution or *in vivo* in the coelom or eye cavity show that a piece of

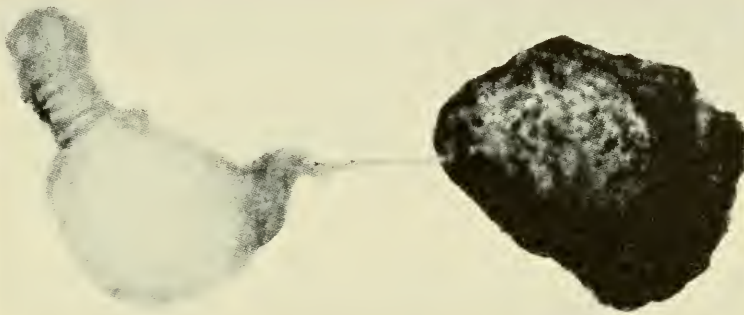


FIG. 160.—An exogastrula with mesentoderm (*left*) somewhat similar in form to an embryo and connected with the irregular ectodermal mass (*right*) only by a slender strand (from Holtfreter, 1933e, *Biol. Zbl.*, 53, H. 7, 8, Thieme).

either may develop as epidermis, neural tissue, or chorda-mesoderm. Holtfreter regards the eye cavity as having complex inductive capacities, but the possibility is not yet excluded that the primary differences in these tissues are nonspecific. The doubtful results of the temperature experiments are not conclusive evidence against this view. In developing as neural tissue ectoderm apparently undergoes a rise in physiological level, relative to the other ectoderm, and apparently a further rise is necessary for development as chorda-mesoderm. The isolated pieces, implanted in coelom or optic cavity, are isolated from the gradient pattern of the embryo and apparently under favorable conditions. Development of chorda-mesoderm from almost all regions of the early gastrula, implanted in the optic cavity, does not seem to be very different in principle from development of a hydranth or head from any level of the hydroid or planarian

body when it is isolated from higher levels of the gradient pattern and sufficiently activated. In the temperature experiments the whole gradient pattern of the parts subjected to the higher temperature is elevated to a higher level, but presumably there is little or no change in the relations of parts and consequently little or no alteration in the course of development.

The conclusion of Goerttler and Lehmann that the anterior region of the presumptive neural plate is more capable of independent differentiation than more posterior levels is in accord with the evidence concerning gradient pattern. This region is the region about the apical pole, the high end of the primary gradient. Under certain conditions its gradient-level is apparently high enough, relative to other parts, to permit independent development as neural plate or neural tissue, while at more posterior levels there must be activation, relative to surrounding ectoderm, either by the inductor or, in isolated pieces, by environmental conditions.

Vogt and Spemann agree in admitting a *Bahnung* toward determination in the presumptive neural plate, independent of the invaginated inductor. But that the determination of presumptive epidermis or neural plate has not become highly specific and fixed, even in the gastrula, is indicated by other results of transplantation. Presumptive ectoderm at the beginning of gastrulation, implanted in presumptive mesodermal or entodermal regions, becomes mesoderm or entoderm (O. Mangold, 1923). Transplanted to dorsilateral regions of older embryos, both presumptive epidermis and neural plate may develop, the neural part ranging from brain with nose and eye to posterior parts of the spinal cord, according to level of implantation. It may also take part in balancer formation or in development of gill or leg, and its deeper layers may become mesoderm. In the pronephric region it may give rise to pronephric tubules; in contact with muscle it may form muscle, this most frequently at more posterior levels, or notochord, or even gut wall (Holtfreter, 1933*b*).

The relative significance of gradient-levels as such and of specific regions or fields, in bringing about these inductions is not known. The question whether tissue of presumptive neural plate can become neural tissue independently of the inductor and of other parts of the embryo appears to have been finally settled by the experiments of Barth (1939*c*). Explants of presumptive neural plate with presumptive epidermis from stages of *Amblystoma punctatum* in which these tissues still form the roof of the blastocoel and before the inductor underlies the presumptive neural region, when fused by their anterior ends, form neural tubes in the region

of fusion. Fusion of the anterior half of such an explant with the anterior end of a whole explant gives similar results. Lateral fusion with antero-posterior axes in opposite directions does not give neural tubes. Moreover, a single explant can develop neural tube if lateral edges are brought together to form a tube having an anteroposterior axis. If anterior and posterior margins are united, a neural tube rarely develops. Barth holds that neural-tube development in these explants results from maintenance of the gradient pattern by proper fusion and healing. If the gradient is obliterated or weakened by the fusions, epidermis, instead of neural tube, results. In view of all the evidence it appears probable that any determination or *Bahnung* toward determination in the presumptive neural plate independently of the inductor is predominantly quantitative and highly susceptible to change in environment.

Presumptive chorda-mesoderm is apparently somewhat more stably determined. Pieces transplanted to other regions of embryos of the same stage as the donor usually invaginate, form chorda-mesoderm, and induce; but under certain conditions this region may take part in formation of ectoderm.¹⁶ The apparently more advanced determination of this tissue is similar to the relatively high stability of high gradient-levels of hydroids, planarians, etc., when transplanted to other regions. They are more able to persist and induce than material from other gradient-levels (chap. xi). The presumptive inductor region behaves in transplants exactly as would be expected if it represents a sufficiently high gradient-level to render it more or less independent of other parts. The question of its metabolism has already been discussed (pp. 153-58).

ORIENTATION OF THE INDUCED EMBRYO IN RELATION TO THE HOST

The longitudinal axis of the neural plate induced from presumptive epidermis by implanted inductor tissue coincides with that of the implant, and this, in turn, is definitely related to the direction of invagination and migration of the chorda-mesoderm material. When invagination in the normal direction in the intact embryo is prevented by injection of gelatin into the blastocoel, it may occur in the opposite direction across the basal region, and the neural plate develops there. Under these conditions part of the inductor region may fail to invaginate, and neural plate may be induced in it; also, part of the presumptive neural plate may become epidermis (Eakin, 1933, 1939a).

The secondary embryonic axis induced by inductor implants is very commonly parallel to the host axis, even when the inductor tissue is im-

¹⁶ Vogt, 1922a; Mangold, 1923; Mangold und Seidel, 1927; Bruns, 1931; Lopaschov, 1935.

planted in different orientation in relation to the host axis.¹⁷ With vitally stained inductor implants, oriented transverse to the host axis, Lehmann observed that invagination began in the direction of the implant axis but changed its direction toward the anterior end of the host. But when the implant axis was directly opposed to the host axis, direction of invagination was highly variable, and the axis of the secondarily induced embryo might be more or less opposed to that of the host embryo. In such cases the secondary neural plate was broad and often did not close normally, and the death rate was high. Apparently there is in the host a directive action of some sort, influencing the direction of invagination and migration of the implanted inductor; and when orientation of implant and induced axis are opposed to the host axis, form and development of neural plate suggests, as Spemann has noted (1936, p. 97), that it is opposed by some factor in the host. In these cases two opposed polarities are involved, one established, the other introduced by experiment. Since the neural plate in normal animals shows a general anteroposterior gradient in early stages, it is probably that the inductor implanted with axis opposed to the host axis induces in some cases a new gradient opposed to that of the host, but this does not attain full development. These results are apparently very much like the relation of the original gradient to the new gradient resulting from section at the proximal ends of stem pieces of *Tubularia* and *Corymorpha*; the proximal hydranth gradient is shorter than the distal, the scale of organization of the proximal hydranth is smaller than that of the distal, and its development is slower. Ganglionic planarian grafts in posterior regions often show similar differences in induced reorganization posteriorly and anteriorly. The alterations in direction of invagination of the amphibian inductor implant by the host are also closely paralleled in ganglionic planarian grafts; the polarity of the graft may be altered to conform with that of the host, particularly with implantation in the head region, but at other body-levels the polarity of the implant usually persists (p. 382). Frequency of induction by *Corymorpha* implants depends on level of origin of implant, level of implantation, and dominance of the host hydranth (p. 378). At present interpretation in terms of gradients involving dynamic factors serves as well for these relations in amphibia as in hydroids and planarians.

HOMEOGENETIC INDUCTION

Induction of a neural plate by chorda-mesoderm is heterogenetic in character, that is, something different from the inductor results from the

¹⁷ Geinitz, 1925c; Spemann, 1931; Lehmann, 1932.

induction. But induction of parts like the inductor—homeogenetic induction—also occurs. Implanted pieces of the dorsal inductor may dominate adjoining host tissue, even of a different species, and induce mesoderm formation from it, with resulting development of a complete chorda-mesoderm; in heteroplastic transplants this consists in part of one species-tissue, in part of another.¹⁸ As noted above, presumptive ectoderm, whether neural plate or epidermis, transplanted to the presumptive mesodermal region, invaginates with it and becomes mesoderm. Here, again, the similarity to dominance in invertebrate reconstitution appears. A piece of inductor dominates adjoining regions of the host, and they become parts of the system concerned. These cases have been regarded as homeogenetic induction, but it may be questioned whether they are really homeogenetic except in the general sense that mesoderm induces mesoderm. The regions induced become parts of the chorda-mesoderm system; but they supplement, rather than duplicate, the parts already present, so that a harmonious whole results; consequently, they must become different in some way, either in gradient-level or specific constitution, from the parts of the system present in the implant. Mesoderm induced by mesoderm from presumptive ectoderm is also capable of inducing neural plate and is not species-specific in action.¹⁹ Presumptive neural plate or epidermis, transplanted to entodermal regions, becomes entoderm. In some of these cases it is perhaps not entirely certain whether or to what extent induction by the region receiving the implant or the general physiological environment is concerned in determining the result.

A case of homeogenetic induction concerning which there is no doubt is the induction of neural plate by neural plate after it has itself been induced by chorda-mesoderm. Not only neural-plate material of the normal embryo but that of a supernumerary neural plate induced by implanted inductor can induce neural plate when implanted in the blastocoel, and its action is not species-specific.²⁰ This result, although unexpected by the investigators (see Spemann, 1936, p. 137), and justifiably so if the inductor is primarily specific in its action, is exactly what might be expected if the inductor is primarily an activator, whatever the manner in which activation is brought about. Neural-plate material has supposedly become different in some way from chorda-mesoderm; but if induction is by means of a specific substance, both must possess or produce it, and neural-plate ma-

¹⁸ Spemann und H. Mangold, 1924; O. Mangold und Seidel, 1927; Lehmann, 1932; B. Mayer, 1935.

¹⁹ O. Mangold, 1923; Spemann und Geinitz, 1927; Holtfreter, 1933*b*; Raven, 1935*b*.

²⁰ O. Mangold und Spemann, 1927; O. Mangold, 1929*c*.

terial of one species must provide the substance necessary to induce neural plate in another. But, if neural-plate induction is primarily activation of ectoderm, plate material, having itself been activated, should be capable of inducing neural plate within a certain range of its developmental stages. Actually the capacity is present not only in the open neural plate but persists after closure of the folds and probably up to hatching. There is apparently no regional correspondence transversely between inducing parts of the plate and the induced plate. The right half of the anterior third of the brain region from an axolotl neurula with closed neural folds, implanted in the blastocoel of a *Triton* gastrula, develops, according to its origin, as a half-brain region but induces a bilaterally symmetrical, very complete head (O. Mangold, 1932a). There is, however, a longitudinal differential in inductions by neural plate. Brain and head structures are induced most frequently by anterior parts of the plate, and, the more posterior the part of the plate used as inductor, the more posterior is the character of the induced structure (O. Mangold, 1933). Different levels of the plate, like different levels of chorda-mesoderm, are apparently "head-inductors" and "trunk-inductors." Also, implants of caudal portions of the plate may bring about development of a supernumerary tail with neural tube and mesodermal somites, induced in host tissue (Bytinski-Salz, 1931; O. Mangold, 1932a). It appears, however, that the extreme posterior part of the plate normally gives rise in part to mesoderm of the tail (Bijtel und Woerdeman, 1928; Bijtel, 1931); consequently, its inducing capacity should be similar to that of caudal mesoderm.

THE PROBLEM OF THE NATURE OF THE INDUCING FACTOR

Early in the course of investigation of amphibian induction by chorda-mesoderm material the same question arose that had arisen earlier with respect to other cases of dominance and induction in development, that is, the question of the manner in which induction is brought about. One line of experiment concerned with this question was the attempt to determine whether other tissues, not only of amphibians but of other animals, would induce. Apparently first was the discovery that the developing amphibian limb bud had some inducing power (O. Mangold, 1928); the regenerating tissue of the urodele leg and tail gave clear-cut induction (Umanski, 1932, 1933). These cases suggest a relation between induction and tissue activity. On the other hand, narcotized dorsal-lip tissue was found to be effective (Marx, 1930). However, the whole problem of induction began to appear in a new light when it was discovered that the urodele dorsal lip and

the neural plate remain capable of induction after killing by heat, freezing, drying, after several hours in 20 per cent HCl, several days in ether, several hours' extraction by ethyl or petrol ether, 6 months in alcohol, followed by impregnation with xylol and paraffin, etc.²¹ This line of experiment was most extensively developed by Holtfreter, using the method of implantation in the blastocoel of a host and that of culture in a modified Ringer solution of pieces of presumptive epidermis placed on, or inclosing, the treated tissue. Equally interesting was the discovery by Bautzmann, and its confirmation and extension by Holtfreter, that regions of the embryo which do not induce when alive can become inductors when killed in various ways. Even fragments of boiled undivided eggs and centrifugates of ovarian eggs are able to induce. It was also found that various adult tissues from many animals and even some plant tissues have more or less inducing power on urodele embryos, some both living and dead, others only when dead.²² Apparently, however, liquid tissue extracts, to be effective, must be coagulated or more or less solidified by addition of substances proved not to be inductors, but solid bodies are not necessarily inductors. The heterogeneous character of these foreign inductors is evident from the following incomplete list, taken in large part from Weiss (1935).

Plants: Cambium of birch, induced small neural plate; growing tip of potato tuber, induction neural of tissue but no distinct plate

Coelentera: *Hydra* tissue, boiled

Annelids (Enchytraeus): Body fragments

Mollusca (Planorbis, Limnaea): Muscles of foot, hepatopancreas

Crustacea (Daphnia): Coagulated body extract.

Lepidoptera (Deilephila): Haemolymph and ganglia of pupa

Odonata (Libellula larva): Fat body, ganglia

Fishes: *Gasterosteus:* heart, liver, ovarian eggs, muscle, spleen; *Danio:* presumptive chorda-mesoderm

Amphibia (Triton, Salamandra, Rana): Liver, heart, ovarian eggs, nuclei and cytoplasm of unfertilized eggs, nuclei more effective, muscle, cartilage, brain, retina, regenerating tissue of tail

Reptiles (Lacerta): Liver, kidney, testis

Birds: Liver, kidney, testis, thyroid, fat body, brain, retina, coagulated chick embryo extract, fragments of primitive streak

Mammals (mouse): Heart, liver, kidney, adrenals, brain lens; also calf liver

Man: Liver, brain, kidney, thyroid, tongue, sarcoma, carcinoma

²¹ Bautzmann, Holtfreter, Spemann, und Mangold, 1932; Mangold, 1932b; Holtfreter, 1933c, 1934a; Spemann, Fischer, und Wehmeier, 1933; Wehmeier, 1934.

²² Fischer und Wehmeier, 1933a, b; Woerdeman, 1933c; Holtfreter, 1934b; Wehmeier, 1934; Hatt, 1934; Waddington and Wolsky, 1936; Ragosina, 1936, 1937; and other papers.

It is sufficiently evident from this list that ability to bring about the change in urodele tissues that constitutes induction is not limited to any particular kind of tissue from any single group of organisms but is a very general property of tissues, either alive or dead or both. Holtfreter concluded from his experiments that induction is effected by a chemical substance or substances. If a single specific substance is the inducing factor in all cases, it must be a substance present in tissues of many kinds from many groups, but the experiments give no clue as to what substance or substances may be concerned. They do not show whether living and dead inductors act in the same way or whether any of the foreign inductors induce in the same way as the natural inductor.

Quite aside from the demonstration of the widespread occurrence of inducing power, these experiments, particularly Holtfreter's extensive data, are of much interest in relation to certain other aspects of the problem of induction. Since certain of the inductors bring about extensive inductions, while others under similar conditions have only slight inducing action, it appears probable that size and completeness of development of the induced structure may indicate, in some degree, the intensity or effectiveness of the inducing action. Frequency of induction also varies with different inductors and may likewise indicate difference in effectiveness. In general, inductors which become such only after killing are apparently weaker in action than the natural inductors. Heating to 100° C. usually weakens inducing action of tissues that induce at lower temperatures, and heating above 120° abolishes it in various cases. Invertebrate tissues are usually less effective than those of vertebrates. Certain tissues—for example, those of the internal glands, such as the liver—are more effective than various others. Almost all the tissues used induce neural tissue, but only certain of them induce mesoderm. The inductions differ greatly in character, ranging from mere epidermal thickenings or sometimes a single lens or balancer to large masses, consisting of various supernumerary organs, several neural tubes being formed in some cases. The neural induction may resemble a part of the spinal cord or may become brainlike; and ganglia, nose, eyes, otic vesicles, and balancers may develop in relation to it. Supernumerary legs may also develop, and induction of muscle and notochord from ectoderm or from mesoderm sometimes occurs.

The apparent absence of any definite relation between character and origin of inductor and character of parts induced suggests that the foreign-tissue inductors are primarily activators rather than definitely specific for particular organs or complexes and that other factors—physiological con-

dition, regions of origin, and developmental stage of ectoderm or other tissue in which induction occurs—play an essential part in determining the result, particularly when pieces of tissue are explanted with the inducing tissue. The effects of foreign inductors are found to be quantitatively greater at anterior than at posterior levels of the hosts. Organs of the head region, brain, sense organs, and balancers appear chiefly in tissue from more anterior levels, while posteriorly atypical neural structures and tails are more frequent; induced kidneys and legs appear more frequently at or near the levels where they normally develop. Also, axial orientation of induced parts, if they are axiate, commonly coincides in direction with longitudinal host axis (Holtfreter, 1934*b*). The anteroposterior differences and the orientation point to the longitudinal or polar gradient of the host as the factor concerned, and the regional differences in character of organs induced may also be determined by this gradient or by the regional or organ fields resulting from it. If this is the case, an adequate activation of a region of the host body within a particular field may be expected to result in development of the organ or organ system characteristic of that field. However, correspondence between expectation and actual character of induced parts is by no means complete. Apparently the fields, if present, are not stably determined, and activation may have different results in the same region. Brain and head structures are said to appear more frequently with strong than with weak inductors. If the inductor is primarily an activator, this is to be expected. A weak inductor may activate only slightly and so induce only some slight differentiation of neural tissue or only a thickening that cannot be certainly identified as neural, even in anterior host regions. Conversely, a strong inductor may activate more posterior levels to such a degree that they develop as brain. Still more intense activation may perhaps induce formation of notochord and mesoderm. It is difficult to account for the experimental results except in some such terms as these, unless we assume specifically different inductor substances for each of the different inductions.

A recent example of such assumptions appears in the work of Chuang (1938, 1940; also further data in another paper, 1940, *Arch. Entw'mech.*, 140). He finds that mouse kidney induces in isolated gastrula ectoderm exclusively brain parts, sense organs, and other head structures and that *Triton* liver induces trunk regions, notochord, muscles, and tail. These differences are regarded as specific effects of the two foreign inductors. With implantation of the kidney or liver in the ventral side of a gastrula these differences in character of inductions are less evident; both

tissues may induce mesoderm and secondary appendages, but in general, mouse kidney induces trunk parts less frequently than *Triton* liver. According to the author, a "host effect" masks more or less completely the specific differences in inducing action of the two tissues. This host effect consists in decrease in frequency of head parts from anterior levels posteriorly and in trunk parts in the opposite direction. Moreover, with mouse kidney the character of induction differs after different periods of boiling: with boiling for a few seconds, induction, especially of mesodermal parts, is increased; after 15 minutes of boiling no mesodermal parts are induced, and frequency of brain inductions is decreased; after 1 hour of boiling there is further decrease in brain inductions, but sense organs and balancers are still induced. These results are regarded as due to different substances or as specific effects of different periods of boiling on some substance or complex. However, the evidence for specific action does not appear any more conclusive in these than in other experiments. The differences may be due to differences in intensity or rate of inducing action and stage of development of host. The "host effect" is obviously an expression of the longitudinal gradient. If the inducing action of kidney and liver were actually specific, how could the host effect alter it to a mere difference in frequency of particular parts? The results after different periods of boiling may also indicate merely different intensities or rates of inducing action, effective at different stages of host development with different reactive capacities of host tissue to the same inducing action.

The conclusion that organ fields of the host determine what parts result from action of foreign inductors (Weiss, 1935) seems not entirely in accord with the data. Very probably the fields are concerned in certain cases—for example, when a lens or a leg is induced—but whether or to what extent the various fields are present and sufficiently developed to determine the result of induction is uncertain. While certain parts, such as brain, appear more frequently in certain regions of the host, they may appear elsewhere. In many cases the region activated by the inductor apparently develops organ fields of its own, as might be expected if fields result from a gradient pattern.

In the light of the evidence considered thus far the question is pertinent whether or to what extent these inductors, natural or foreign, living or dead tissues, are actually organizers? It was pointed out above that the longitudinal gradients of ectoderm and inductor coincide in direction after invagination and more or less closely in extent. Does the chorda-mesoderm do anything more in normal development than "reinforce" and per-

haps steepen the primary ectodermal gradient? Assuming that the inductor does act in this way, it is only indirectly concerned in organization in that it alters the gradient pattern in the ectoderm, that pattern being the real organizer. The chorda-mesoderm itself develops longitudinal and transverse organization, but in its earlier stages there is no definite evidence of anything more than a gradient pattern in it, and with differential inhibition the higher levels of this pattern, that is, anterior and median regions, are most inhibited.²³ Implanted into other than the normal position, it may sometimes induce a new gradient, as in the case in which the induced embryo is more or less opposed in orientation to the host. Here the inductor gradient determines the gradient pattern of the induced embryo, and so its organization, and comes nearer being a true organizer than in normal development; but even here, according to this view, the gradient pattern induced in the ectoderm is the real organizing factor.

The foreign inductors, if living, may or may not possess gradient differences; whether living or dead, their action is usually more or less local and does not induce an orderly whole but merely certain tissues or organs in more or less unordered complex. If these inductors are primarily activators, the character of the induced part depends chiefly on two factors—level of host gradient in which it arises and degree or intensity of activation. Either of these may be predominant in determining the result. These inductors, however, give us no information as to the manner in which organization occurs. Weiss (1935) admits this as regards foreign inductors but regards the natural inductor not only as activator but as organizer. Actually, however, there is often much organization in the complexes induced by foreign inductors, but it is not normal. If these inductors are not organizers, the organization must result from pattern of some kind already present in the part subjected to inductive action, and the only sort of pattern that seems adequate to account for the varied results is a gradient pattern, wholly or predominantly nonspecific regionally. But, as already noted, foreign inductors can probably determine new gradient patterns in the host tissue; if so, they are indirectly organizers.

Needham, Waddington, and Needham (1934) hold that two factors, "evocation" and "individuation," are concerned in induction. Evocation consists in bringing about development of an embryonic axis in the ectoderm; individuation, in determining the regional character of that axis. The foreign inductors—living, dead, or extracts—are, in general, only evocators. This conception of induction is open to certain criticisms.

²³ See pp. 257-65; also Bellamy, 1919; Lehmann, 1937*a*; Cohen, 1938*b*.

First, there is the interesting question: What is the nature of an embryonic axis without regional character? Second, the foreign inductor does not always evoke an embryonic axis; it may induce merely a mass of tissue or an unordered complex; and when an axis does appear, it usually coincides with the host axis. Another interesting question is: How can a piece of some tissue—for example, liver—induce an embryonic axis? Third, according to these authors, an axis without individuation may range from a neural tube with notochord and somites in proper position, but one end not distinguishable from the other, to histologically differentiated neural, chordal, and mesodermal cells without definite order. But development of neural tube with somites and notochord in normal relations represents a very definite axiate pattern. Even if the two ends are not distinguishable as different regions, it probably did not arise all at once; and if it did not, a pattern with regional character of some sort must have been present. The concept of an embryonic axis without regional character seems somewhat metaphysical. Does not an axiate pattern, partial or complete, constitute individuation?

The concept of an individuation field originating from the inductor in normal development (Waddington and Schmidt, 1933) also presents difficulties. The inductor does not originate embryonic pattern; it is a part of that pattern. In normal development it does not determine the polar axis of the embryo but merely plays a part in determining certain developmental events in relation to that axis. Does not the pattern of the whole amphibian egg constitute the individuation field, within which orderly changes precede and bring about invagination and induction?

CHEMICAL ASPECTS OF THE INDUCTOR PROBLEM

That induction may be due to a chemical substance was suggested by Spemann and Mangold (1924). The later discoveries that dead tissues and tissue extracts, when associated with a more or less solid carrier, can induce, and that boiling, treatment with alcohol, ethyl ether, acetone, acetone followed by several days in water, or glacial acetic acid, does not destroy inducing power and in some cases increases it, seemed to indicate that any inducing substance present in the material is not soluble with these treatments, though it must presumably reach the overlying ectoderm in some way. Further experiment showed, however, at least for acetone, that both the extracted residue and the acetone extract could induce.²⁴

²⁴ Spemann, Fischer, und Wehmeier, 1933; Fischer und Wehmeier, 1933*a*, *b*; Wehmeier, 1934; Holtfreter, 1934*a*, *b*.

According to Woerdeman (1933*a*, *b*, *d*), the glycogen content in the apical hemisphere of the amphibian blastula is high and more or less uniform but decreases in the dorsal lip region as it invaginates. Similar observations were made by Tanaka (1934). In transplanted pieces of the dorsal lip the same decrease occurs on invagination (Raven, 1933*a*, 1935*a*). On the basis of his observations Woerdeman advanced the view that decrease in glycogen in the inductor region at the time of gastrulation may be associated with induction, presumably through glycolysis. He found also that implants of malignant tumors known to have high glycolytic activity induce (Woerdeman, 1933*c*). Fischer and Wehmeier (1933*a*; Wehmeier, 1934) also suggested, independently of Woerdeman and on other grounds, that glycogen might be an inductor substance and obtained neural-plate inductions with glycogen from liver, but in later experiment they found that inducing power decreased to zero as purification of the glycogen preparations became more complete (Fischer und Wehmeier, 1933*b*). Induction was also obtained with the unsaponifiable material from ethereal extracts of crude glycogen.²⁵ Both Woerdeman (1933*c*) and Holtfreter (1934*b*) found glycogen inactive. More recently the conclusion has been drawn from experiments with glycogen from rabbit liver that an agent associated with glycogen is a highly effective inductor (Heatley, Waddington, and Needham, 1937). Pasteels (1936*c*), using a different method from that of earlier workers for demonstrating intracellular glycogen, maintained that the supposed decrease in the region of the dorsal lip does not occur and that, consequently, increased glycolysis cannot be responsible for the inducing power of the chorda-mesoderm. However, with still another method Heatley and Lindahl (1937) found that during gastrulation glycogen decreases in all parts of the embryo but that decrease is greatest in the invaginating material. The high anaerobic glycolysis of the inductor region (three times that of other parts) and its high respiratory quotient (about unity), indicating carbohydrate metabolism, have already been noted (pp. 154-55). However, according to Brachet (1939), carbohydrate metabolism is not necessary for induction; induction may take place in the presence of agents inhibiting glycolysis. He suggests that proteins may be important factors in induction. Evidently still further investigation along these lines is necessary. Whatever the final conclusion, the glycogen hypothesis is interesting, as pointed out by Weiss (1935, p. 664), in that it suggests that induction is mediated by

²⁵ Waddington, Nowinski, Needham, and Needham, 1934; Waddington, Needham, Nowinski, Lemberg, and Cohen, 1936.

metabolic activity with glycogen as source; that is, the activity, rather than the substance, is the real inductor.

Other attempts were made with other methods to isolate a particular inductor substance. The material of neurulae, crushed in a small volume of water and centrifuged, separated into three layers—the cellular debris, an aqueous layer containing protein but not entirely fat-free, and a fatty layer. Induction of neural tubes was obtained by implantation of coagulated parts of the aqueous, protein-containing layer. Also, neurulae, ground with anhydrous sodium sulphate, the mass extracted with ethyl ether or petroleum ether and the extract mixed with solid carriers shown by experiment not to be inductors, gave, when implanted, neural tubes or, more commonly, solid rods and other masses, “probably neural in character.” Petroleum ether extracts of adult amphibian viscera were also inductive. From these experiments the conclusion was drawn that the inductor is a definite chemical substance, soluble in ether and probably lipoidal.²⁶

Continuing work with ethereal extracts, Waddington, Needham, and co-workers obtained induction in *Triton* and axolotl with the unsaponifiable fractions of ether extracts of whole newt bodies and mammalian liver and with the parts of these fractions precipitated by digitonin, implanted after emulsifying in egg albumin and coagulating by heat. Minute doses of certain synthetic polycyclic hydrocarbons, massive doses of certain acids, and certain other substances may also induce. Recently methylene blue has also been found to induce, and it has been suggested that it acts by setting free an inductor substance. Nuclei of unfertilized amphibian eggs are more potent inductors than the cytoplasm. The earlier suggestion of these workers was that induction was due to a sterol-like substance; but further experiment seems to indicate that, even if such substances are inductors, other substances also induce.²⁷ The possibility that methylene blue, a respiratory catalyst in certain concentrations, induces directly by activation may be noted. Methylene blue in low concentrations increases oxygen uptake 45 per cent in amphibian embryos, in high concentrations is inhibitory; and Janus green and neutral red in low concentrations also

²⁶ Waddington, Needham, and Needham, 1933*a*, *b*; Needham, Waddington, and Needham, 1934.

²⁷ Waddington, Needham, *et al.*, 1934, 1935; Waddington and D. M. Needham, 1935; Waddington, Needham, *et al.*, 1936; Waddington, Needham, and J. Brachet, 1936; Waddington, 1938*a*, *b*. For a quantitative study of induction by a water-soluble hydrocarbon see Shen, 1939. See also Needham, 1939, “Biochemical aspects of organizer phenomena,” *Growth*, Suppl.

induce neural development in ventral gastrula ectoderm isolated into the solution.²⁸

It was found by Fischer, Wehmeier, and associates that pure fatty acids of animal and plant origin, also synthetic acids, emulsified in agar and implanted, induce and that exhaustive extraction of amphibian embryos or tissues of other animals with aqueous or organic solvents does not abolish inductive power. Moreover, the nucleoprotein fractions derived from these tissues are the active agents.²⁹

Induction, owing either to cephalin or some impurity, was obtained with cephalin fractions of mammalian brain (Barth, 1934*c*). However, acetone extracts of brain, containing sterols but no cephalin, and the unsaponifiable fraction of cephalin also induce. Cephalin preparations that induce also produce more or less cytolysis; and other cytolytic agents—for example, digitonin—with acid or alkaline buffers, also induce. The protein residue of calf brain is more potent than the lipoid extract.³⁰

Since various tissues induce after killing but not while living, and certain foreign inductors—for example, cephalin and digitonin—produce more or less cytolysis in the host, it has been suggested by Barth and others that this cytolysis of host cells may set free inducing substance. Neural induction by fuller's earth, silica, and CaCO_3 has been reported by Okada (1938). These substances do not give off chemical inductors, but they do produce injury and cytolysis of tissues. Okada regards substance or substances set free by the cytolized tissue as the inductor in this case. Induction by microcautery in the blastocoel is reported by Cohen (1938).

Evidently the chemical problem of induction is by no means solved. If induction or "evocation" is primarily activation, it seems not at all improbable that many chemical substances may be inductors and that irritation may also induce. It is also by no means certain that any of the foreign inducing factors is identical with the natural inductor. It has not even been demonstrated that the natural inductor is a substance. In any case, it is not evident how a particular chemical substance can organize a neural axis unless its concentration or the amount set free is graded in the inductor, but such a gradation provides an axial differential independent of the chemical constitution of an inducing substance. In any case, induction by a particular chemical substance, whether produced by the natural inductor or of other origin, throws no light on the problem of or-

²⁸ Beatty, De Jong, and Zielinski, 1939.

²⁹ Fischer, Wehmeier, Lehmann, Jühling, und Hultzs, 1935.

³⁰ Barth, 1934*d*, 1937, 1939*a*; Barth and Graff, 1938.

ganization. For organization a spatial pattern of some sort is essential, and it is not at present evident how a sterol or other particular substance can, of itself, originate an orderly and definite spatial pattern. The problem of organization is still the problem of the spatial pattern in the embryo and any other developmental system. In the amphibian the natural inductor does not originate this pattern but is a part of it and plays a role in modifying it. The foreign inductors merely modify it by local activation or otherwise. The spatial pattern, whether we call it a gradient, a gradient system, or something else, is the real organizer. In this connection Waddington's recent discussion of organization (see p. 455, footnote 8) is of particular interest because of his inability to throw any real light on the problem.

INDUCTION IN EARLY DEVELOPMENT OF OTHER CHORDATES

ASCIDIANS

The ascidian egg was earlier regarded as an extreme "mosaic," but recent experiment has shown that a considerable capacity for reconstitution is present (p. 577). Moreover, according to the most recent work with isolated blastomeres, an inductor is concerned in early development. The posterior blastomeres of the four-cell stage and the apical blastomeres of the eight-cell stage are found to be incapable of independent differentiation when isolated, but the anterior basal blastomeres of the eight-cell stage can differentiate independently and are necessary for the differentiation of other parts. These blastomeres represent presumptive notochord, nerve cord, entoderm, and some mesoderm, but ascidian anterior half-embryos differ from amphibian dorsal half-embryos in that they do not reconstitute (Rose, 1939).³¹

According to other experiments, removal of various parts of the ascidian embryo some time before gastrulation gives no certain evidence of induction (von Ubisch, 1940, and another paper, 1940, *Arch. Entw'mech.*, 140). After removal of prospective notochord material the nervous system and other organs are normal. After removal of all prospective muscle and most of mesenchyme other organs develop without evidence of induction by mesoderm. When prospective neural material is removed, no nervous system develops; evidently there is no neural induction in other ectoderm

³¹ Comparison with amphibian development raises the question whether the two sides of the egg and embryo designated by Conklin (1905a) and others as anterior and posterior are not more nearly dorsal and ventral; neural plate and notochord develop from the "anterior" basal cells of the eight-cell stage.

by chorda-mesoderm or any other part. After removal of entoderm there is apparently some reconstitution of entoderm, probably from ectoderm; and entoderm may perhaps have some inductive action on the papillae of attachment. Rose's data suggest induction at a very early stage, but how far it is similar to amphibian induction is not yet evident; conceivably, it may have taken place before the stage of von Ubisch's experiments.

FISHES

The posterior part of the teleost embryonic shield, representing the dorsal lip of the blastopore, possesses inducing capacity, as in amphibians. When pieces of it are transplanted to other regions, even the extraembry-

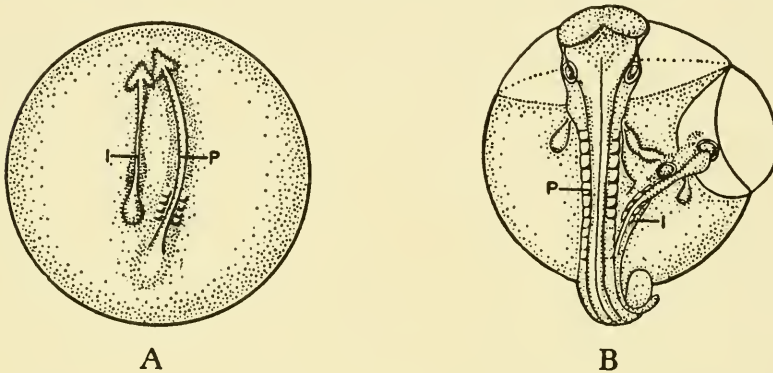


FIG. 161, A, B.—Induction in the teleost, *Fundulus*. A, induced embryo (I) in primary embryonic shield; B, induced embryo (I) outside primary shield (from Oppenheimer, 1936c).

onic regions of the blastoderm, they may induce secondary embryos.³² When transplantation is within the embryonic shield, the induced and the primary embryo are closely similar, and corresponding parts are at the same levels; but embryos induced by transplants to extraembryonic regions show no definite relations to the primary embryo, and corresponding parts may develop at different levels (Fig. 161). The polarity of the embryo induced in the extraembryonic region may even be opposed to the host polarity. Evidently, within the embryonic shield the longitudinal pattern of the host determines direction of the induced secondary embryonic axis and the levels at which its organs develop. In extraembryonic regions gradient pattern is slight or absent; consequently, the inductor gradient may become the chief or only factor in determining axial direction. However, blastomeres explanted to salt solution may undergo more

³² Oppenheimer, 1934, 1936a, b, c; W. Luther, 1935, 1936a, b.

or less differentiation without gastrulation (Oppenheimer, 1936*b*). Moreover, up to the end of the blastula stage a piece from any region of the blastoderm, including part of the margin, may, when isolated in the yolk sac of an older individual, give rise to a more or less complete embryonic primordium. Neural tissue develops in most cases, notochord almost as frequently, even from extraembryonic regions containing none of the presumptive chorda-mesoderm; and very frequently gut and kidney also develop. This capacity of parts of the blastoderm for independent reconstitution decreases rapidly in extraembryonic regions with gastrulation, and inductor action becomes necessary for embryo formation (Luther, 1936*a*). In its high capacity for reconstitution in earlier stages the teleost blastoderm differs from the amphibian blastula, although under certain conditions neural, or even mesodermal, tissue may develop from presumptive amphibian epidermis or neural plate, as noted above. Luther regards the inductor as an activator, differing quantitatively, not qualitatively, from other parts, and holds that the capacity of isolated pieces of the blastula blastoderm to reconstitute embryonic primordia results from levels of activity high enough in all regions at this stage to determine embryo formation without aid of an inductor. As gastrulation begins, this activity decreases in extraembryonic regions, and embryo formation occurs there only by induction. This view agrees closely with that suggested above for the amphibia, and the data on differential susceptibility in teleosts also show a high susceptibility in the inductor region as gastrulation approaches (p. 150). Perhaps the point of greatest interest in these experiments on teleosts, as compared with those on amphibians, is the reconstitution of new inductor regions in isolated pieces, even from extraembryonic regions without any of the original inductor. This does not suggest any considerable specificity in the inductor. The fact that the extraembryonic regions do not develop inductors and embryos in normal development suggests that the dominance of some other region, presumably the more active embryonic region, or later the inductor region itself, prevents such development, and that an activation resulting from isolation is sufficient to bring about development of neural tissue or even of inductor region. In short, the data give further support to the conception of neural induction as primarily an activation and appear difficult to interpret in other terms.

Following division of the archenteric roof of the trout embryo into anterior, middle, and posterior pieces, inclosure of each in indifferent ectoderm from the extraembryonic region, and implantation in the yolk sac,

the anterior piece induces no neural tissue in the ectoderm, the middle piece induces brainlike parts, the posterior piece chiefly spinal neural structure and occasionally otic vesicle. The middle piece is regarded as "head organizer," the posterior piece as "trunk organizer" (Eakin, 1939*b*); but the experiments do not show that there is anything more than a quantitative difference between them.

BIRDS

Following discovery of induction by the region of the dorsal blastopore lip in amphibians, the question whether a comparable induction occurred in the chick naturally arose. It was suggested that the region of the node at the anterior end of the primitive streak is an "organizer," or at least essential to formation of the embryo.³³ However, it was found that portions of the streak not including the node can continue development when isolated *in vitro*, and implantation experiments showed that not only the node region of the streak but more posterior regions without any part of the node can induce neural tissue or plate or a more or less complete embryonic axis. Implants from more anterior levels of the streak differentiate into notochord, somites, and neural tissue; those from levels farther posterior form only mesodermal structures; but both induce. Both anterior and posterior derivatives of the streak, the head process, and the sinus rhomboidalis, and also the neural plate after its formation, can induce neural plate.³⁴ The action is not species-specific; heteroplastic implants between duck and chick are effective. Pieces of primitive streak killed and coagulated by boiling water also induce. The host "individuation field" (Waddington) influences axial orientation of induced parts, as in amphibians; but sometimes the induced axis is opposed to that of the host and is then supposedly determined by the inductor. Apparently there may be considerable reconstitution of the graft into more anterior parts or a larger part of the longitudinal axis than would have been formed in normal development. Reaction of the host to implanted inductor decreases as development progresses. Neural plate is induced up to a later head-fold stage, but at this stage only by implantation in anterior regions; at mid-trunk levels only epidermal thickening results. In neural-fold stages only thickening occurs at all levels, and at early somite stages the epidermis shows no reaction (Woodside, 1937).

³³ Wetzel, 1925*a, b*, 1929; T. E. Hunt, 1929, 1931, 1932; Willier and Rawles, 1931.

³⁴ Waddington, 1930, 1932, 1933*b*, 1934, 1935, 1937; Waddington and Schmidt, 1933; Waddington and Taylor, 1937.

Pieces of extraembryonic ectoderm, presumptive epidermis, and presumptive axial or lateral mesoderm of primitive-streak stage implanted under the primitive streak of the same stage are all capable of developing into neural tissue. Their anteroposterior axes may be altered or reversed by the host, but dorsiventrality persists (Abercrombie, 1937). The neural development of these implants is considered to be due to induction by the host streak. The polarities of pieces of primitive-streak pieces implanted beneath primitive streak may also be reversed by the host and their regional character altered to conform to host-level. In some cases the host axis shifts to one side of the implant, and the latter induces either extra neural tissue or a new axis, or the implant may be incorporated in the host body (Abercrombie and Waddington, 1937). By implantation into the primitive streak presumptive ectoderm may be converted into mesoderm (Waddington and Taylor, 1937). In these cases the effect of the host on the implant is evident in varying degree. The implant may retain its individuality and induce, or be altered as to polarity and body-level, or become a part of the host body.

An interesting apparent induction by the entodermal axis has been obtained by separating epiblast and entoderm in early primitive-streak stages of duck and chick embryos and replacing them with longitudinal axes opposed (Waddington, 1933*a*). Effects on development of this procedure vary. In cases of axial induction two embryos may develop with heads together and longitudinal axes opposed, one representing the epiblast axis, the other apparently induced by the entodermal axis; or the epiblast axis determines an embryo, the entodermal axis a transitory primitive streak. In still other cases the original primitive streak disappears, and the embryo determined by the entodermal axis persists. Even when a new embryonic axis is not induced, the original axis may be inclined toward the direction of the entodermal axis or be semicircular, or the embryo may be very short. Waddington holds that the entoderm merely induces the cell migrations that result in development of the primitive streak but admits that a gradient system or other axiate pattern is necessary to account for the orderly character and definite directions of the migrations. However, it does not appear from the data that the entodermal axis differs essentially in its inducing action from implanted parts of the primitive streak. It is apparently a rather effective inductor, for it is able to determine a new polarity opposed to the original. That this polarity results from the imposition on the epiblast of a new gradient pattern corresponding to the entodermal pattern seems probable. The persistence of

both or either one of the two axes doubtless depends on incidental factors, levels of activity of epiblast and entoderm, and perhaps their alterations by operation; it suggests varying relations of dominance of one or the other axis.

Neural-plate induction in the chick has also been obtained by implantation of the anterior part of a two-somite rabbit embryo (Waddington, 1934), affording still another example of the nonspecific character of these inductions.

However, it appears from many experiments that neural tissue can develop independently of an inductor in the chick. In various isolation experiments forebrain has been found to develop independently of notochord, and grafts of notochord have proved ineffective as inductors.³⁵ Moreover, explants of purely ectodermal pieces from anterior regions of very early primitive-streak stages may give rise to neural tubes (Rudnick, 1938b).

MAMMALS

The few data available show that neural induction can occur in a mammalian embryo but give no information as to the part it plays in normal development. Chick primitive streak implanted in the rabbit embryo cultivated *in vitro* may induce neural-plate formation, but thus far implantation of rabbit primitive streak in rabbit embryos has given only uncertain results as regards induction, although the implants may differentiate into neural tissue and notochord (Waddington, 1934, 1936b, 1937).

NEURAL INDUCTION: CONCLUSIONS AND QUESTIONS

From the data it appears that many different substances can induce, directly or indirectly, development of neural tissue in amphibians and that under certain conditions neural tissue may develop without an underlying inductor. It may perhaps be questioned whether there is a specific chemistry of induction in the sense that a particular substance or group of substances is the inducing factor. It also appears that the natural inductor does not originate axiate embryonic pattern but is a part of it, and that the pattern, either of the inductor or of the reacting ectoderm or both, rather than the induction itself, is the real organizer. Induction in vertebrates throws no light on the essential problem of organization, the problem of the origin and nature of the pattern within which the inductor acts.

The question whether neural induction is primarily an activation or a specific action, or in normal development an orderly axiate series of spe-

³⁵ T. E. Hunt, 1931; Waddington, 1932, 1933; Wetzel, 1936.

cific actions, has been raised and discussed above. With the progress of experiment evidence for specific action of the inductor seems progressively less conclusive, even though specific substances may be concerned. The data on neural induction do not conflict with the evidences of gradient pattern given by other lines of experiment. On the other hand, they may be regarded as throwing some light on the manner in which development proceeds from gradient patterns of certain sorts.

An interesting question in relation to the problem of organization is that of the origin of the inductor region. In amphibian embryos under experimental conditions a region of invagination may appear in other than the normal position, but evidently in relation to a pattern (pp. 259, 429). These cases suggest that in normal development the dorsal inductor may originate within a more general pattern, perhaps primarily as a local activation, perhaps as the earliest determination or differentiation; but how its localization on one side of the egg is determined is not known (p. 686). That the inductor region is not a primary feature of pattern is also indicated by the appearance of new inductors in reconstitution of isolated extraembryonic parts of the fish blastoderm. The problem of organization in vertebrates involves not merely the results of inductor action but the origin and pattern of the inductor and the origin and nature of the pattern within which it appears and acts.

The question of the role of induction in normal development is not fully answered as yet. In the teleosts isolation of a part of the blastoderm is sufficient to bring about development of new embryonic axes in the entire absence of the inductor. This being the case, does the inductor play any essential part in normal neural development? Under certain conditions explants of presumptive amphibian epidermis can develop into neural tissue or even into mesoderm in absence of the inductor, though such development probably results in some cases from activating conditions or substances which perhaps should be regarded as inductors. As regards birds and mammals, we know at present little more than that new embryonic axes may result from implantation of certain parts.

If induction is essential to the development of a morphological axis in the ectoderm and if it is primarily an activation, it is an activation in a definite graded axiate pattern, and this is the first step toward axiate determination and differentiation. Induction by the chorda-mesoderm does not represent the origin of axiate pattern but is merely an expression of pattern already present. That cells or regions at different relative levels of physiological activity behave differently in development appears cer-

tain. In various hydroids hydranths degenerate and stolons develop, even from apical, as well as from basal, ends, in standing water, in KCN, and with other inhibiting agents, while in flowing, well-aerated water hydranths develop (pp. 172-75). Is this fundamentally different from development of presumptive amphibian epidermis as epidermis under certain conditions, as neural tissue, or as mesoderm under others?

LENS INDUCTION

Among the organ inductions of later embryonic stages that of the lens by the optic cup in amphibians was known before induction of neural plate by chorda-mesoderm and has been the subject of much experiment and discussion.³⁶

DEVELOPMENT OF THE AMPHIBIAN LENS

In the anuran map (Fig. 150) the presumptive lens region is at some distance from the region of the optic primordium, but in the course of development the optic cup comes to lie beneath the presumptive lens ectoderm. Under natural conditions the lens develops from the epidermis as the optic cup comes into contact with it. The amphibian optic vesicle arises as a hollow lateral outgrowth from the developing brain and remains attached to it by a stalk. Its wall, at first with little regional difference in thickness, becomes, in part, the thick retinal layer, in part the much thinner tapetum; the retinal layer becomes concave lateroventrally, forming the optic cup, and lens formation occurs by a proliferation and thickening of the inner layer of the epidermis overlying the concavity of the optic cup. As the concavity becomes deeper, the developing lens extends farther into it and separates from the epidermis. Earlier stages of lens formation are indicated in Figure 162. This course of development very naturally suggests that the optic cup may have something to do with lens development. This is also indicated by the fact that, in cases of approximation of eyes and cyclopia, lenses develop in normal position with respect to the optic cup, but presumably from other epidermis than the presumptive lens-forming region. Decisive evidence concerning the role of the optic cup in lens formation has been sought by various lines of experiment: replacement of presumptive lens epidermis by other epidermis, removal of optic vesicle preceding lens formation, implantation of optic primordium in other regions, etc.

³⁶ For fuller discussion of the data than is possible here and for the literature see O. Mangold, 1931a; Spemann, 1936, 1938.

EXPERIMENTAL LENS INDUCTION IN DIFFERENT SPECIES

In the earliest experiments with *Rana fusca* as material neither lens development nor the loss of epidermal pigment characteristic of corneal development occurred when the presumptive optic region was removed at the stage of early neural plate, and a similar dependence of lens formation

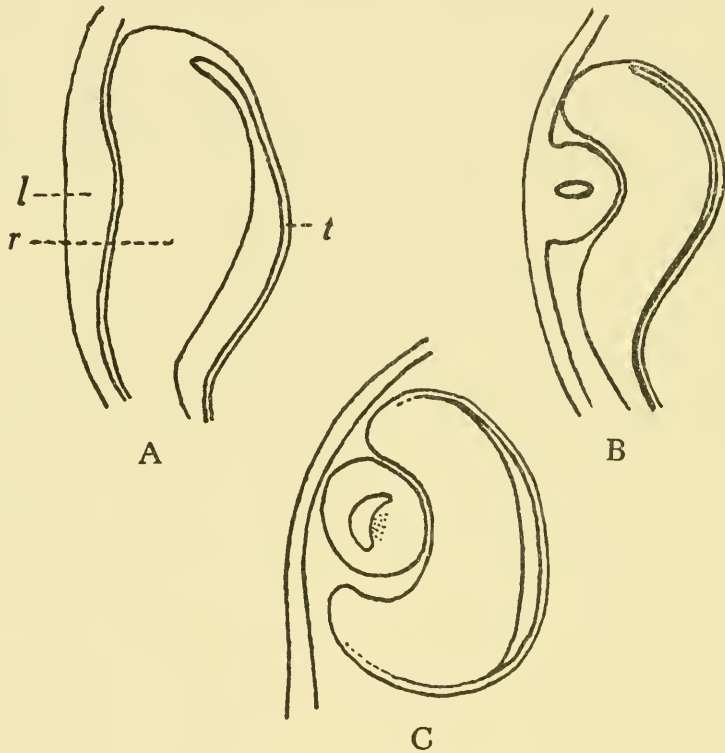


FIG. 162, A-C.—Stages in development of amphibian lens. A, early optic cup; r, retinal; t, tapetal layer; and l, epidermal thickening of early lens development; B, C, later stages, in C formation of fibers beginning (schematized after Rabl, 1898).

on presence of optic cup in other vertebrates seemed probable (Spemann, 1901a). But lack of uniformity soon became evident: lenslike structures were found to develop in *R. palustris* after killing the optic primordium (King, 1905); further experiment showed that a well-formed lens with fibers may develop in *R. esculenta* after removal of the optic region from the neural plate; also, in *Bombinator*, *R. fusca*, and *R. catesbiana* apparent beginnings of lens development (lentoids) sometimes appear in similar

experiments.³⁷ No indications of lens development in absence of the optic cup have been observed thus far in the urodeles, *Pleurodeles* (Pasquini, 1927), *Triton taeniatatus*, and *T. alpestris* (O. Mangold, 1931a); and in *Amblystoma* results of earlier experiments were negative or only slightly positive (Le Cron, 1907). Another form of experiment likewise indicates dependence of lens formation on presence of optic cup in *Triton*. Removal of the head mesoderm in the early neurula usually results in approximation of the eyes to the median plane or in complete cyclopia, and lenses develop in normal relations to the optic cups. This experiment is regarded as doubly significant: first, because the condition determining cyclopia occurs at a relatively late stage of development; second, because the cyclopia results from a local mesodermal defect, while in cyclopia produced by inhibiting chemical agents, or occurring in the reconstitutions resulting from ligature in early stages, the whole embryo is affected, and the locus of the presumptive lens region, as well as position of the optic cup, may conceivably be altered. When the optic primordium is reduced in size, either by reconstitution from a part or otherwise, the lens of *R. esculenta* is not correspondingly reduced (Spemann, 1912b), but in other forms for which data are available there is more or less close correspondence in size of optic cup and lens.³⁸

Transplantation experiments bearing on lens formation may consist in (a) transplantation of presumptive lens epidermis to other regions of the body; (b) transplantation of optic vesicle or cup to other regions or explantation with epidermis from other regions; (c) substitution of other epidermis for presumptive lens-forming epidermis. All these experiments have been performed on at least some amphibian species, some of them on several species.

Transplantation of presumptive lens epidermis of *Amblystoma punctatum* to other head regions in early and late neurula results in lens formation in the new location (Harrison, 1920); but other species, including *R. esculenta*, have given negative results (Spemann, 1912a). Transplantations of the optic region of the neural plate (optic plate) or the optic vesicle of a later stage to other body regions have been made in various species with induction of lens. The transplanted optic primordium develops and differentiates to an advanced stage, and transplanted pieces may reconstitute to optic cups of small size. Substitution of other epi-

³⁷ Spemann, 1907, 1912a, b; von Ubisch, 1923, 1924, 1925c, 1927; Pasquini, 1931.

³⁸ W. H. Lewis, 1907a, b; Spemann, 1912a; Wachs, 1919; von Ubisch, 1924; Filatow, 1925; O. Mangold, 1931a.

dermis for that normally overlying the eye by reversing the anteroposterior axis of head epidermis, by transplanting epidermis from other regions of head or trunk, or by regeneration of epidermis from adjoining regions after removal of the presumptive lens epithelium has also given positive results in many species.³⁹ Regenerating tissue of urodele leg and tail and of anuran tadpole tail transplanted to the eye may form a lens after removal of the original lens (Schotté and Hummel, 1939). Even optic vesicles explanted with skin pieces may induce a lens in other than the presumptive lens epidermis (Perri, 1933).

As the data stand at present, lens can be induced in head epidermis not immediately adjoining the eye region and in trunk epidermis by the optic cup in *R. fusca*, *R. sylvestris*, *R. temporaria*, *R. catesbiana*, *R. ridibunda*, *R. pipiens*, *Hyla arborea*, in trunk epidermis, probably also in head epidermis but data are lacking, and in *T. taeniatus* and *Pleurodeles wallii*. According to earlier experiment (Spemann, 1912a), lens induction occurred only in head epidermis in *Bombinator pachypus* and *R. esculenta*, but more recently it has been obtained from both head and trunk epidermis in both species. Earlier experiment on *A. punctatum* gave positive results only with epidermis adjoining the presumptive lens epidermis, but Stone and Dinnean (see footnote 39 below) report lens induction in ventral ectoderm.

In general, reactivity appears to be less in trunk than in head epidermis, particularly in regions near the eye. Lenses induced in trunk epidermis are usually smaller and less developed, and apparently less frequent, than in head epidermis, suggesting a relation between the effect of induction and position of the epidermis in the anteroposterior gradient. Although nasal and otic primordia of *Hynobius* are capable of independent differentiation, the optic cup can induce lens in them by overcoming whatever determination exists (Ikeda, 1937).

Mangold and Spemann regard the data as indicating that in some species—for example, *R. esculenta* and *A. punctatum*—the presumptive lens region is determined as lens before the optic cup comes into contact with the epidermis, while in others it is not so determined or is less determined, and that, in general, capacity of other epidermis to react to the

³⁹ W. H. Lewis, 1904, 1907a, b; Spemann, 1905, 1912a; Ekman, 1914; Harrison, 1920; Filatow, 1925a, 1934; Beckwith, 1927; Pasquini, 1927, 1932; von Ubisch, 1927; Gostejew, 1935; etc. Mangold, 1931a, p. 263, gives in tabular form results obtained with different species on lens formation by other than the presumptive lens epidermis. See also Stone and Dinnean, 1940, "Origin of the lens by induction in the salamander, *Amblystoma punctatum*," *Proc. Soc. Exp. Biol. Med.*, 40.

optic cup by lens formation is less limited in those species in which the presumptive lens region is not already determined, and vice versa. Von Ubisch, on the other hand, maintains that all amphibian species are essentially alike at corresponding developmental stages, as regards lens determination.⁴⁰ It seems that final conclusions concerning this point must await further investigation. Inhibiting effects of the operative procedures on transplanted epidermis or optic primordium or on the host tissues, rate of healing, and various other factors may differ with different material and in different experiments. Since reactivity of the epidermis to the inductor decreases and becomes regionally more limited as development progresses, slight difference in stages used by different experimenters may be responsible for some of the differences in results. Inductive intensity of optic cup may differ in different species; in some it may be sufficient to induce lens formation only in more anterior levels, in others, from any level. Epidermal reactivity also appears to differ regionally, and the anteroposterior differential may be greater in some species than in others. It is generally agreed that the optic cup influences the lens after the original inducing action. The lens primordium, or the lens after fiber development has begun, may develop somewhat further after removal of the optic cup, when placed in the blastocoel of a gastrula or transplanted to other regions; but sooner or later its structural pattern is lost and it degenerates.⁴¹

Recent experiments have placed the whole question of lens potency and its restriction in a new light. Optic primordia implanted in regenerating tissue of tadpole or adult urodele tails can induce lens from the regenerating cells. Regenerating leg tissue of urodele or regenerating tail tissue of urodele or anuran tadpole transplanted to the lensless eye of an adult urodele or a large anuran tadpole can give rise to a lens.⁴²

In other experiments a Nile blue stained ectoderm from the side of a larva is transplanted to the eye of a larva from which all corneal and orbital epidermis has been removed. Later implanted epidermis is removed from over the eye, permitting regeneration from the Nile blue stained edges of the implant, and lenses or lentoids may develop from the regen-

⁴⁰ For a discussion of this point see O. Mangold, 1931a, pp. 288-89. Spemann, 1936, pp. 24-57, and 1938, chap. iii, discusses lens induction at length with numerous figures and references.

⁴¹ Le Cron, 1907, *Amblystoma*; Fischel, 1917, *Salamandra*; Filatow, 1925c, *Triton*; Krüger, 1930, *Triton*.

⁴² Schotté, 1937, 1938; Schotté and Hummel, 1939.

erated tissue (Schotté, 1940). Unless Nile blue has spread from cells of the implant to those of the host and host cells are involved in the regeneration, it appears that the regenerating flank ectoderm from a hatched larva is capable of developing lens under inductive action of the eye. Potency for lens formation, apparently lost in the course of development, may reappear in cells undergoing reconstitution. This seems to be a case of de-differentiation and indicates clearly the relative character of so-called "determination."

THE QUESTION OF SPECIFICITY IN AMPHIBIAN LENS INDUCTION

In amphibian lens induction, as in neural induction, there is no tissue-, organ-, or species-specificity. Nonlentogenous epidermis of *Bufo vulgaris* transplanted over the optic cup of *R. esculenta* develops lens. Heteroplastic transplantations between *Triton* species and between *Triton* and axolotl also give positive results (Mangold, 1929c; 1931a, p. 277). In heteroplastic transplantations between *Triton* species with lenses of different size the induced heteroplastic lens maintains, in general, donor size, though it may sometimes be smaller in consequence of inhibiting conditions. The optic cup undergoes more or less adjustment to lens size, apparently through influence of the lens on its growth (Rotmann, 1939).

Various tissues, living or dead, can induce lens—for example, boiled posterior neural plate, entodermal cells killed by alcohol, fresh liver, and boiled heart of salamander (Holtfreter, 1934a, b). In *Triturus pyrrhogaster* nasal primordia, otic vesicle, brain, heart, liver, neural plate, dorsal archenteric wall, ectoderm, mesoderm, and entoderm of head region implanted in place of optic primordium induce lens in the presumptive lens epithelium (Okada and Mikami, 1937). The optic cup can induce other organs than lens. Implanted below the skin of regenerating tails of large frog tadpoles, it may induce lens, also olfactory parts, otic vesicle, and mouth cavity (Schotté, 1937). In this case the optic cup apparently induces something resembling the head region in the regenerating tissue; doubtless the high level of activity and the slight degree of differentiation in the regenerating tissue are concerned in the result.

A chemical substance produced by the optic cup or other tissue has commonly been supposed to be the lens-inductor, but the positive results with transplantations between species and genera and lens induction by various tissues, living and dead, raise the same questions as regards specificity of the inducing factor that arose concerning neural induction. To what extent activity-level of epidermis or attainment of a certain degree

of specificity dependent on developmental stage and differing in different species and at different body-levels may be concerned in reaction of epidermis by lens formation to an optic cup or other living or dead inductor is not known, and practically nothing is known concerning intensity or degree of inducing power of optic primordia or of the effects of operative procedures, transplantation, explantation, etc., upon it.

ORIGIN OF PATTERN IN THE LENS

The lens possesses a polarity normally coincident in direction with the optic axis. Mangold (1931*a*, pp. 272-74) regards the optic cup as determining this polarity but holds that in lens developing independently of the optic cup the polarity is already determined in the lens primordium. Spemann (1936, p. 56) believes that the polarity is determined by the inducing action from one side. Since the lens in early stages is essentially similar to a bud, its polarity may result from this form of development rather than from the induction itself. The central region of the bud, presumably the most active region in early stages, becomes the lens proper, the peripheral regions forming the capsule. Instead of becoming an elongated axis like most buds, direction of growth of the originally central region appears to undergo reversal in direction and to be directed toward the cavity of the lens primordium and the epidermis, whether because of pressure on the retina or some other factor (Fig. 162). In any case it is evident that a polarity may originate in relation to the differential activity represented in the differential growth of the lens primordium and quite independently of an inductor. The lens primordium buds from the epidermis, and the lens proper buds into the interior of the lens vesicle formed by separation of the bud from the epidermis, but the axis of the lens is in the same direction as that of the original bud, though direction of growth is reversed. The only relation of an inductor to the lens polarity may be that of initiating a region of activity grading off from a center. Alterations of lens polarity under experimental conditions offer no difficulties to this conception. Experiments by Dragomirow (1929) show alteration in relation to other developing parts than an optic cup. Early optic cups with presumptive lens epidermis were so transplanted that the epidermis was between the developing otic primordium and the optic cup. Lenses developed with two axes at differing angles to each other, sometimes opposed, one in the usual relation to the optic cup, the other vertical to the surface of the otic vesicle. Here the effect of the otic ves-

icle may be merely a differential activation, decreasing radially from the region most affected.

The lens fibers extend between the two polar regions, forming concentric layers; at each pole they come together along a line, forming a short suture (Fig. 163). In the amphibian

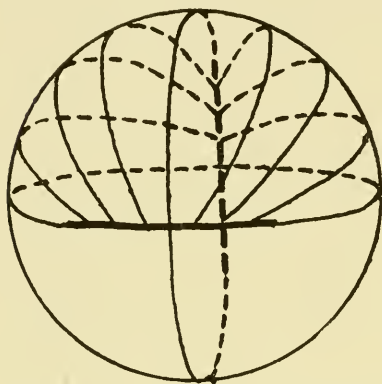


FIG. 163.—Diagrammatic, indicating course of lens fibers and in heavier lines the two sutures in which the ends of the fibers meet (after Rabl, 1898).

eye the inner suture, toward the retina, is anteroposterior, the eye being regarded as lateral; the outer, dorsiventral in direction. If presumptive lens epidermis, optic plate, or early optic vesicle is turned 90° , it is possible, up to a certain stage, to obtain lenses normal in relation of sutures to the optic cup; but during closure of the neural folds, that is, before the lens develops at all, the suture pattern is fixed and not altered by turning (Woerdeman, 1934). Apparently, then, although it may be altered by an inductor up to a

certain stage, it is determined in relation to the general pattern quite independently of the inductor, perhaps in relation to dorsiventral or anteroposterior gradient pattern or both.

LENS DEVELOPMENT IN OTHER VERTEBRATES

Lenses appear frequently, either in the normal location or in other head regions without relation to eyes or contact with nervous tissue, in teratological embryos of teleost fishes, both those occurring occasionally in nature and those experimentally produced. They may even develop in anophthalmic forms, and several supernumerary lenses may be present in an individual; they vary in size and degree of development but may be large and advanced in differentiation.⁴³ It seems beyond question that some of these lenses develop from other than presumptive lens epidermis. The factors concerned in their origin are not known, but their development suggests a condition in the epidermis associated with stage of development and region of body rather than with action of a specific inductor. According to Werber, however, blastolysis, that is, a dissociation of parts of organ primordia; occurred in consequence of exposure to inhibit-

⁴³ Mencl, 1903, 1908; Gemmill, 1906a, b; Stockard, 1909, 1910b, c; Werber, 1916a, b, 1918.

ing chemical agents, in his experiments chiefly to butyric acid and acetone. The free lenses were assumed to be induced by fragments of the optic primordia scattered through the head region in consequence of blastolysis; and the hypothesis that the vertebrate lens is induced in all cases was advanced, but conclusive evidence supporting it is lacking. Lenses also appear in normal relation to the optic cups of teratological forms when these reach the epidermis, but experiments demonstrating induction seem to be lacking. That the normally lentogenous teleost epidermis can develop lens in absence of optic cup is evident from some of these teratological forms, and there is little doubt that the supernumerary lenses develop from other epidermis. The teratological forms concerned are differential modifications of development. It may be suggested that in those experimentally produced the differentially inhibiting action of the agent decreases or perhaps abolishes the differential in the head region. With the activation associated with recovery following return to water, the integrating and ordering factors in the head region being less effective than normally and different regions being more alike, local activations in the epidermis at certain stages, either induced by underlying parts or perhaps originating in the epidermis, may bring about formation of several lenses independent of eyes. The appearance of supernumerary adventitious polarities in *Corymorpha* with essentially similar treatment may be recalled (pp. 416-17).

Data bearing on lens induction in the chick are few, but it has been shown that in stages from primitive streak to four somites the optic cup can induce lens in ectoderm of head, neck, or trunk, in later stages only in head and neck. Up to five lenses or lentoids may develop in relation to one transplant (Alexander, 1937). Earlier experiments also show or indicate induction in the normally lentogenous or adjoining epidermis.⁴⁴

OTHER INDUCTIONS IN VERTEBRATE DEVELOPMENT

As development progresses, other inductions of organ systems and organs, most extensively studied in amphibians, take place in particular parts of the body or under experimental conditions at certain developmental stages. Some of these have been mentioned in connection with the field concept (chap. viii), and certain others are briefly considered here.

RETINA AND CONJUNCTIVA

Although retinal and tapetal parts of the optic primordium become visibly different in early stages (Fig. 162, A), the tapetum may still give

⁴⁴ Danchakoff, 1924, 1926; Reverberi, 1929; Willier and Rawles, 1931; Rawles, 1936.

rise to retina when brought into contact with epithelium of the developing otic vesicle (Dragomirov, 1937; Ikeda, 1937). The thinning and loss of pigment of the epidermis in development of the conjunctiva is induced by an optic vesicle or cup, by a fragment of the retina, or by a lens. Development of conjunctiva from epidermis of other regions can be induced, either by transplantation of optic vesicles or by grafting other epidermis over the optic primordium, provided there is contact between the two.⁴⁵ Moreover, the reaction is not species-specific, at least not for various urodele species (Mangold, 1929c; Harrison, 1929a), and a limb bud implanted in place of the optic cup at certain stages apparently induces conjunctival development (Dürken, 1916), though this has been questioned (H. Petersen, 1924).

THE EAR

Pieces of hindbrain or medulla implanted near the ear region or even in the flank induce development of otic structures; this induction is effective between *R. pipiens* and *A. punctatum* in either direction.⁴⁶ Presumptive medulla, but not forebrain or spinal cord, implanted in regenerating tissue of the tadpole tail, induces vesicles resembling otic vesicles (H. S. Emerson, 1939). The ability to induce ear is itself induced in presumptive hindbrain by the underlying mesoderm, and this mesoderm can induce ear development directly in foreign ectoderm in absence of hindbrain (Harrison, 1935). The optic primordium induces otic vesicles among other organs in regenerating tadpole tail tissue, but perhaps only indirectly (Schotté, 1937). After removal of the presumptive otic region the ear may develop from regenerated ectoderm or from ectoderm transplanted from other body regions and even from anurans to *Triton*.⁴⁷ On the other hand, the earlier stages of ear development from presumptive otic ectoderm of neurulae are apparently independent of other organs, but induction is necessary for full differentiation (Kaan, 1926). If this is true, the ear is primarily determined as a locus in the general pattern of the ectoderm without induction; but induction can determine ear development in ectoderm of other regions, even in different species, genera, and orders of amphibia, raising once more the question of specificity of the inducing action. If otic induction is primarily an activation, it is activation at a certain period of development when the reacting tissue has doubtless attained a condition different from that of early stages. The same induc-

⁴⁵ Fischel, 1900b, 1917, 1919; Spemann, 1901a; W. H. Lewis, 1905; Groll, 1924.

⁴⁶ L. S. Stone, 1931; Albaum and Nestler, 1937; Ponomarewa, 1936.

⁴⁷ *Amblystoma*: Tokura, 1925; Kaan, 1926; Yntema, 1933; Harrison, 1935, 1936, anura to *Triton*: G. A. Schmidt, 1936c.

ing factor may apparently have different effects on the same ectodermal region at different developmental stages.

The general gradient pattern of the body is apparently a factor in the results of heterotopic transplantations. The farther ventral or posterior to the otic region the place of origin of ectoderm transplanted to the otic region, the less otic development takes place. Transplants of presumptive otic ectoderm develop more completely at a level between ear and eye than at levels some distance posterior and ventral to the otic region, and the most complete development at the anterior level occurs at an earlier host stage than at the more posterior and ventral levels (Yntema, 1933). The anterior level apparently attains a condition favorable to, or less inhibitory to, ear development earlier than the posterior and ventral levels. These data certainly suggest definite relation to the anteroposterior and dorsiventral gradient pattern. The differences in otic development in ectoderm from other than the otic region have commonly been regarded as indicating differences in potency, but they may be merely differences in susceptibility or reactivity to the inducing factor. Otic development in regenerating tail tissue, following implantation of optic primordium or presumptive medulla, suggests, first, that the regenerating tissue has undergone a considerable dedifferentiation and possesses, or has regained, potencies supposedly lost, and, second, that even here different embryonic regions may serve as inductors.

It is generally agreed that the otic vesicle induces aggregation and flattening of mesenchyme cells about itself and that these give rise to the cartilaginous capsule. That this mesenchymal reaction is not primarily a specific determination and may be altered by change in conditions is emphasized by Filatow (1927). He regards it as not widely different from the inflammatory reaction following implantation of a foreign body or, in later stages, even of an otic vesicle. Under influence of an implanted otic vesicle primordia of other organs—a vertebra (Yntema, 1933), and in the fish, *Acipenser*, shoulder girdle (Filatow, 1930)—show more or less capsular reaction. However, it appears that the capsular reaction may be absent when otic primordia are transplanted to other than the normal otic region.⁴⁸ This induction may be chemical in character, as far as accumulation of mesenchyme is concerned; but, in view of the wide occurrence of cartilage development, it can scarcely be regarded as specific for the otic vesicle, except perhaps mechanically in determining form of the capsule.

Development of the amphibian tympanic membrane is normally de-

⁴⁸ L. S. Stone, 1926; Luther, 1927; Balinsky, 1929; Kaan, 1930; Yntema, 1933.

pendent on the annular tympanic cartilage. When this is removed, the membrane does not develop; when it is transplanted to other regions, membranes form over it. This induction occurs in any region of the skin before and after metamorphosis. The transplanted quadrate will induce tympanic membrane, and the suprascapula shaped into annular form is probably slightly inductive (Helff, 1938; 1934a, b).

Skin of fully developed dermal plicae with mucous and other glands from postmetamorphic *R. palustris* transplanted to the backs of larvae of *R. catesbiana* undergoes complete regression of the plicate structure to generalized skin. Retransplanted after this regression to the otic region of metamorphosing *R. clamitans* or *R. catesbiana*, it forms fully differentiated tympanic membrane (Helff, 1934c). Whether, or to what extent, mechanical or chemical factors, activating or specific, are concerned here is not yet evident.

According to the data, three successive inductions are involved in development of the amphibian ear: the primary neural induction, induction of the otic vesicle by hindbrain regions, and induction of the capsule by the vesicle; probably other inductions are involved in later development of its parts. But, as in case of the lens of at least some amphibians and apparently of the neural plate, some degree of definition or determination as the locus of particular developmental events occurs in the presumptive otic region in relation to the general pattern of earlier stages before these inductions take place. If that general pattern is a gradient pattern, the otic region is primarily defined by its gradient co-ordinates, and induction by hindbrain or other inductor may be merely an acceleration or intensification of changes already initiated by position in the gradient pattern. The inductor, according to this view, is primarily an assisting factor in bringing the reacting region to a physiological level at which a certain kind of differentiation, already initiated, can continue to a certain result. As inductor and reacting region differentiate, there may be definite specific relations between them.

BALANCER, ORAL REGION, GILLS

Early larval stages of most urodeles possess so-called "balancers," slender, elongated, tentacle-like organs, slightly club shaped at the tips, consisting of epidermis and mesodermal core derived from the neural crest, and situated ventral and somewhat posterior to the optic region. From early neurula stages of *Amblystoma* on, presumptive balancer ectoderm can develop a balancer when transplanted alone to other head regions,

but not until later stages when transplanted to the trunk. This difference is attributed to the mesoderm of the head region (Harrison, 1925*b*). The anterior part of the archenteric roof placed in the blastocoel of the early *Triton* gastrula comes to lie ventrally and induces balancer there. Although the axolotl possesses no balancer or only traces, inductor transplants to *Triton* may induce balancer development (Mangold, 1931*b*). Heteroplastic transplants of presumptive balancer ectoderm from *A. punctatum* to the corresponding region of *A. tigrinum*, which has no balancer, may develop balancer; but ectoderm of *A. tigrinum*, transplanted to the balancer region of *A. punctatum*, does not give rise to balancer (Harrison, 1925*b*). Heteroplastic transplants of presumptive balancer ectoderm and of ventral ectoderm to the balancer region between *Triton* species give balancers characteristic of the donor species. The reaction, but not the inducing factor, is species-specific (Rotmann, 1934, 1935*b*). Neural crest material of *R. fusca* in the blastocoel of *Triton* and axolotl may induce neural plate and various modifications in the host, among them often supernumerary balancers, but always in the balancer region (Raven, 1931, 1933*b*).

The urodele mouth has small teeth, the anuran mouth horny jaws; in anuran larvae two suckers are present ventral to the mouth, but no balancers. The entoderm underlying the presumptive oral ectoderm is necessary for formation of the oral region in *Triton* and axolotl. Presumptive oral ectoderm of neurulae, transplanted or explanted alone, does not form a mouth (Stroer, 1933). Presumptive abdominal ectoderm of the anuran gastrula, transplanted to the urodele mouth region, gives rise to an anuran mouth with horny jaws and suckers; abdominal urodele ectoderm in the anuran mouth region develops a urodele mouth with teeth, and, if the transplant includes the balancer region, balancers, sometimes supernumerary, develop. Both balancer and sucker may develop in some cases, one from the host, the other from the donor ectoderm; and, if the boundary between urodele and anuran ectoderm is in the region of the anuran sucker, part of a sucker develops on the anuran side, none on the urodele side, the development ceasing sharply at the junction.⁴⁹

Presumptive anuran gill ectoderm, transplanted alone to other regions in neurula or tail-bud stages, develops gill stumps, and the gill pattern in transplants turned 180° is similarly turned. Ectoderm adjoining the gill region is also capable of gill development (Ekman, 1913*a, b*; 1922).

⁴⁹ Spemann und Schötté, 1932; Schötté, 1932, 1933; Rotmann, 1935*a*; Holtfreter, 1936; G. A. Schmidt, 1937*a*.



Transplanted gill ectoderm of *Amblystoma* shows less evidence of gill development (Harrison, 1921*b*), but after removal of gill ectoderm and mesoderm and implantation of a limb bud in the gill region some gill development may occur in ectoderm adjoining and usually ventral to the gill region (Detwiler, 1922). After removal of presumptive branchial entoderm in tail-bud stages the gill does not develop, and this entoderm, transplanted beneath ectoderm and mesoderm slightly posterior and ventral to the gill region, can determine gill development (Severinghaus, 1930; Ichikawa, 1934, 1938). Induction of gill development may occur in presumptive neural plate or in ventral ectoderm transplanted heteroplastically to the gill region between *Triton* species (Spemann und Rotmann, 1931), or from axolotl to *Triton* (Rotmann, 1935*c*), or even xenoplastically, from an anuran, *Bombinator igneus*, to *Triton* (G. A. Schmidt, 1937*b*). The induced gills show donor characteristics more or less clearly in time of appearance, size, time of degeneration, and, when differences between donor and host are considerable, by lack of correspondence between gills and underlying mesentoderm. However, some modification by the host of size and perhaps of period of persistence apparently takes place. In general, the experimental results indicate that presumptive branchial ectoderm of the neurula and tail-bud stages possesses some capacity for independent gill development, apparently less in urodele than in anuran species; but for complete gill development the underlying entoderm or mesentoderm seems to be necessary.

"DOUBLE ASSURANCE" IN AMPHIBIAN DEVELOPMENT

Implanted pieces of the dorsal lip can induce development of a neural plate from ectoderm entirely outside the presumptive neural region, and induction by the underlying chorda-mesoderm is supposedly a factor in neural-plate formation in normal development. However, as we have seen, there is considerable evidence that the presumptive neural plate, or at least its anterior part, is in some degree "determined," predisposed to the course of development resulting in neural plate, and under certain conditions can undergo more or less development along this line independently of an underlying inductor. Similarly, lens development can be induced in ectoderm of various regions by an optic cup; but in some amphibians a lens can develop in the presumptive lens region quite independently of an optic cup, and in others some degree of determination or predisposition toward lens formation is apparently present in the presumptive lens epidermis. These and other cases of apparently twofold determination Speemann regards as examples of the principle of double assurance or the

synergetic principle in development.⁵⁰ Development of the part is doubly assured, on the one hand, by its own determination and, on the other, by the inductor. It must be pointed out, however, that there is no double assurance unless the part concerned can actually develop independently of, as well as in consequence of, the action of the inductor. As far as we know, a complete, normal neural plate does not develop independently of an inductor. The neural tissue or neural tube developing independently of an inductor could not serve an individual as a central nervous system. Its independent development is without value to the organism. The lens of some amphibian species apparently constitutes a better example of double assurance, but in other species double assurance is apparently lacking. In the teleost embryo under inhibiting conditions several lenses may develop independently of an eye, seemingly a multiple assurance but without significance for the normal organism.

But even if it be granted that both independent differentiation and development by induction are concerned in certain parts, the principle of double assurance throws no light on the problem of the organization which initiates independent development or of the factors concerned in induction. It is a teleological, not a physiological, principle. We must, then, ask the question: What does double assurance mean physiologically? Does it mean anything more than that the pattern within which a greater or less degree of independent development or determination takes place and the inductor act on the part concerned in very much the same manner, though perhaps in different degree or intensity? Assuming, for the moment, that the primary pattern of organization is a gradient pattern, the presumptive neural plate represents a certain region, a certain range of levels, in that pattern; and whatever independent development is possible results from its position in, and relation to, that pattern. The inductor apparently represents a higher range of gradient-levels and, by raising the level of presumptive neural plate, makes possible further development. But apparently almost any stimulating or irritating factor acts in some degree like the natural inductor, though those factors are usually without axiate pattern, and any axiate pattern developing must be of ectodermal origin. These suggestions do not necessarily involve the assumption that the invaginated chorda-mesoderm differs only quantitatively from the presumptive neural plate. It may already be specifically different when

⁵⁰ Spemann, 1931, pp. 508-10; 1936, p. 59; 1938, p. 92. For earlier biological applications of this principle see Rhumbler, 1897; Braus, 1914. For the principle of *kombinative Einheitsleistung*, a somewhat broader concept than double assurance, see Lehmann, 1928, and later papers cited in Bibliography.

it invaginates, but the data of neural induction do not support the view that specific characteristics of the chorda-mesoderm are directly concerned in determining that overlying ectoderm shall become neural tissue. They may play a part in determining features of the pattern of its further development and differentiation; but other tissues, living or dead, from many organisms, tissue extracts, and residues and various synthetic substances can induce development of neural tissue from ectoderm. Again the question arises: Is this induction anything more primarily than activation? If this induction is not specific, the chorda-mesoderm is only indirectly neural inductor; it activates ectoderm, and the activated ectoderm becomes neural tissue. Later in development, when ectoderm has attained a somewhat different condition, an activation may result in development of a lens, at least in head ectoderm. The epidermis has very probably undergone some degree of differentiation which makes lens development possible with sufficient activation by an inductor or otherwise.

PRESENT STATUS OF THE INDUCTOR PROBLEM

By way of conclusion to this chapter the attempt is made to indicate briefly the conception of induction to which the evidence, at present available, points. It is impossible to draw a hard and fast line between what is quantitative and what is specific in living protoplasts; but, according to the evidence considered in preceding chapters, axiate organismic pattern in its early stages appears to be predominantly or wholly a quantitatively graded pattern, always, of course, in a protoplasm of specific constitution. Nonspecific alterations of that pattern alter the course of development. Even a difference in oxygen tension may determine the physiological and morphological differences between a stolon and a hydranth in certain hydroids (pp. 172-75).

In general, the natural inductors of early stages are, or at certain developmental stages become, high gradient-levels, and their inducing action apparently consists in alteration of condition in regions representing lower levels. This alteration evidently involves activation; and, with the progress of experimental analysis, it appears increasingly probable that from the hydroids to the vertebrates the primary factor in the inductions of early development, whether reconstitucional or embryonic, is activation. Amphibian head-, trunk-, and tail-inductors, for example, represent different gradient-levels of the inductor region; and there is no conclusive evidence that at the time of gastrulation they differ in other ways, though they may already be different from the ectoderm. Moreover, their induc-

tive effects are not strictly regionally specific but show gradations depending on gradient-levels in the reacting ectoderm as well as in the inductor region. The head-inductor may induce trunk; the trunk-inductor, head; and between a normal head and a trunk without head there is a graded series of defective heads, as in the planarian, though not of the same types. Moreover, the great number and variety of inductors of neural tissue in amphibians does not support the view that the inductor is a specific chemical substance with a specific effect; in fact, this possibility seems to be excluded. If neural induction is primarily a localized, non-specific activation of ectoderm, effectiveness of many different inductors is to be expected and seems to have been demonstrated.

In so far as an inductor is a nonspecific activator, it is not directly an organizer or organization center. The natural inductor is itself a product of pattern and organization, and as inductor it alters pattern relations in other parts; but the pattern thus altered is the real organizer or reorganizer. The inductor comes nearer to being a real organizer when it determines an entirely new pattern, as in hydroids and planarians, where the region activated by section at the proximal end of an isolated piece or a small graft determines a new polarity at right angles to, or opposed to, the original polarity. In these cases the inductor apparently acts solely as activated region without pattern and determines a gradient pattern on a smaller or larger scale. Even here, however, the activated region or graft is only indirectly organizer through the gradient pattern. When an implanted piece of the dorsal lip induces in a vertebrate an axis in a different direction from, perhaps opposed to, the host axis, this is not entirely new but is apparently an imposition on the ectoderm of the axiate pattern of the inductor, and this pattern is the organizer.

Even if the earliest inductions in development are nonspecific activations or begin as such, it does not follow that all inductions have this character. With progress of differentiation induction may become more specific, both as regards inducing factor and reacting system. Certain hormones are evidently inducing factors. They are specific substances produced by specifically differentiated organs and producing certain effects on other specifically differentiated organs, but even in some of these cases the question whether the effect is primarily an activation of the reacting organ or direct determination of specific differentiation is open. In any case, these specific inductors of later stages are scarcely to be regarded as organizers. Their effects result from presence of a specific organization, not from its absence.

CHAPTER XIII

CERTAIN EMBRYONIC RECONSTITUTIONS IN RELATION TO PRE-EXISTING PATTERN

MANY of the regional differences in reconstitution of isolated embryonic parts are evidently expressions of the axiate pattern already present and therefore of interest in relation to the problem of its nature. This chapter is concerned with some of these, particularly in forms with relatively high capacity for reconstitution in earlier stages.

COELENTERATES

Although embryonic stages and planulae of this group should be interesting in this connection, experimental data are few. According to Zoja (1895), hydroids may develop from $1/2$, $1/4$, $1/8$, and even from $1/16$ blastomeres of certain forms, but nothing is known concerning differences in capacity in relation to the original pattern. However, if $1/8$ and $1/16$ blastomeres can develop into planulae that give rise to hydroids, there is probably little or no regional differentiation in the pattern. How a $1/16$ blastomere attains the pattern of the whole is an interesting question. In view of the instability of polarity in the embryo and planula of *Phialidium* (pp. 167, 425), it seems possible that in some of these isolated blastomeres a new polar pattern may be determined by the differential between free surface and surface in contact.

In some other forms pieces of undivided eggs above a certain size and $1/2$ and $1/4$ blastomeres can become wholes, but $1/8$ blastomeres resulting from equatorial division become abnormal larvae or show little development.¹ Pieces of some hydrozoan planulae give rise to hydroids (Maas, Child), but pieces of planulae of the anthozoan *Renilla* do not develop beyond the swimming stage. These fragmentary data throw little light on the question of the original pattern in these forms. Limitation or absence of reconstitution in $1/8$ blastomeres or in planula pieces suggests some degree of polar differentiation but may perhaps be due to scale of polar organization too large for the blastomere or piece rather than to

¹ Maas, 1901, 1905, 1908; E. B. Wilson, 1903*b*; Conklin, 1908.

restricted potency. Further experiment with developmental stages of this group is necessary for definite conclusions.

ECHINODERMS

Some of the earliest experiments on embryonic reconstitution were performed with sea-urchin eggs and cleavage stages, and their high reconstitutive capacities aroused great interest and led to extensive further experimentation. These early experiments appeared particularly significant because they came at a time when the predeterministic Weismannian theory of development was current and when studies of cell lineage in annelids and mollusks indicated a high degree of predetermination in those forms.²

Early stages of sea-urchin development were regarded as constituting a harmonious-equipotential system by Driesch (1899, 1901), but with the progress of experiment some data seemed to point to a different conclusion. In early experiments parts of sea-urchin eggs and blastomeres were isolated by section or by shaking, and it was shown by various experimenters (Boveri, Driesch, Loeb, Morgan, Delage, and others) that nucleated and even nonnucleated fragments of unfertilized eggs above a certain size could be fertilized with sperm of the same or of another species and might develop and that whole eggs and nucleated fragments could be activated to parthenogenetic development by various agents. These experiments, however, gave little or no information concerning developmental pattern because the portion of the egg represented by a particular fragment was usually not known. They did indicate, however, that considerable reconstitution was possible. In recent years development of techniques of isolation of blastomeres and of local vital staining has made it possible to obtain much more exact information concerning regional differences in reconstitution in these eggs and embryos than earlier methods permitted. Single blastomeres and groups can be isolated as desired, locally stained for orientation or identification, and transplanted in desired locations and combinations.³

² In view of the more definite results obtained in recent years with improved techniques, discussion of the earlier work appears unnecessary. Among the numerous workers who contributed to this field, the following are mentioned with dates indicating approximately the periods covered by their papers, but the papers are given in the Bibliography only when particularly referred to in the text. Driesch, 1891-1923; Boveri, 1889-1914; Morgan, 1894-1908; Ziegler, 1896-1925; Garbowski, 1904-10; Jenkinson, 1911*b*; von Ubisch, 1913—; Runnström, 1914—. For bibliography to 1928 see Schleip, 1929.

³ See, e.g., von Ubisch, 1925*a*, *b*, 1929, 1932*a*, *b*, 1936; Hörstadius, 1928*a*, *b*, 1935, 1936*a*, *b*, 1937*a*; Hörstadius und Wolsky, 1936; also pp. 436-46.

Experimentation along these lines has given results of much interest in relation to the problem of pattern, though it has not yet provided a basis for complete agreement as to the character of that pattern. Discussion of the regional differences in reconstitution in relation to the axiate pattern of the sea-urchin egg is based largely on the extensive experiments of Hörstadius from 1928 on. Here again, use of the terms "animal" and "vegetal" will be convenient in certain connections. Also, it will be recalled that, according to the data on differential susceptibility and differential dye reduction, the apical or "animal" pole is the high end, the basal or "vegetal" pole the low end, of the primary polar gradient. According to the Runnström hypothesis, animal and vegetal poles are, respectively, the regions of highest concentrations of animal and vegetal substances.⁴

Animal halves, the eight mesomeres of the sixteen-cell stage, or $an_1 + an_2$ of a later stage (Fig. 145 [p. 438]) represent about two-thirds of the presumptive ectodermal region. They usually give rise, when isolated from basal halves, to apical partial forms without entoderm or mesenchyme but with variation in scale of organization from the extreme apical type, with the long cilia normally characteristic of the apical region extending over most of the surface, to less extreme forms (Fig. 146, *A-E* [p. 439]). Isolated an_1 rings, that is, approximately the most apical fourth, become extreme apical partial forms, usually completely covered in earlier stages with the long cilia and later uniformly ciliated (Fig. 147, an_1 [p. 441]). Reconstitution of isolated an_2 rings is less extremely apical and very similar to that of the apical half (Fig. 147, an_2 [p. 441]). These isolated apical halves and an_1 and an_2 are more apical in reconstitucional development than in normal development. They may develop as extreme apical regions, and some of them are apparently almost or entirely apolar or anaxiate. According to Hörstadius (1935), this is because the animal gradient gains the upper hand over the vegetal; but how this takes place is not made clear. Presumably concentrations of hypothetical animal and vegetal substances are not immediately altered by isolation. What determines the assumed alteration? The results suggest that some effect of regions farther basal is present in normal development of the whole embryo but absent in the isolated apical parts. The observed primary polar gradient, decreasing basipetally, and the activation in the basal region preceding gastrulation, giving rise to a secondary gradient opposite in direction to the primary and partly obliterating it (pp. 134-40) provide, a somewhat different basis for interpretation of the experimental results.

⁴ See pp. 241, 243, and chap. vi in general.

In the isolated apical half, or an_1 or an_2 , the primary gradient is not partly obliterated or reversed by the secondary, resulting from basal activation; consequently, the scale of polar organization becomes larger than in normal development and larger than the apical half or the fourths, and they develop as more or less extreme apical partial forms. These apical partial forms are essentially similar in origin to the apical partial forms in the reconstitution of *Tubularia* and *Corymorpha* pieces. Short pieces of these forms with a single gradient reconstitute unipolar apical partial forms which usually have a much larger scale of organization than bipolar partial forms developing from pieces of the same length with two opposed gradients (Fig. 116 [p. 346]). The later the developmental stage at which isolation of apical halves or fourths occurs, the less is the change in scale of organization from normal development (Hörstadius, 1936a). This, of course, is to be expected if there is a secondary basal activation and alteration of the primary gradient.

That the apical overdevelopment of apical halves and fourths is really due to the large scale of organization determined in the isolates, rather than to restriction of potency for entoderm and mesenchyme, is shown by the fact that after temporary exposure to differentially inhibiting conditions (lithium salts) apical halves may develop primary mesenchyme and entoderm, gastrulate, develop skeleton, and become plutei; or in some cases they may even become exogastrulae (von Ubisch, 1925a, b, 1929; Hörstadius, 1936a). Runnström and Hörstadius regard lithium as exerting a specific effect in increasing the vegetal gradient or substance, but it has been shown that after the secondary basal activation occurs lithium produces modifications in the opposite direction and that other agents besides lithium produce entodermization (pp. 228–33). Exposure to lithium salts involves two factors—the direct, differentially inhibiting action, and the recovery after return to water, which may also be differential. In early stages lithium inhibits the higher apical levels of apical halves more than the basal levels; consequently, scale of organization of these regions is decreased and extent of extreme apical development is therefore less than without the inhibiting action. Basal regions of the apical halves are also inhibited to a lesser degree, but sufficiently to bring them down to the gradient-levels of presumptive entoderm and mesenchyme in early stages, that is, entodermization of presumptive ectoderm results. With recovery after return to water apical dominance is inadequate to prevent the secondary basal activation, and mesenchyme formation and gastrulation take place much as in the normal individual. With sufficient inhibi-

tion exogastrulation may result. According to this suggestion, the effect of lithium in these cases is merely lowering of the levels of the primary gradient and decreasing dominance of the apical region, so that the basal activation is possible within the smaller scale of organization. The decrease in scale of these apical halves is quite similar to the decrease in scale by inhibiting conditions in the reconstitution of *Tubularia* and *Corymorpha* pieces. Pieces giving rise to apical partial forms in well-aerated water may develop as complete individuals after certain degrees of inhibition. At present the evidence for a specific effect of lithium on a vegetal substance or gradient does not appear convincing. Apical halves of sea-urchin embryos occasionally reconstitute entoderm and mesenchyme and gastrulate in normal environment, according to von Ubisch (1936a, b). He suggests that this takes place only under the most favorable conditions, but in the light of other experimental evidence it appears probable that such development results from slightly inhibiting conditions producing lower levels of metabolism and consequently smaller scale of organization.

Reconstitution of isolated basal halves is essentially similar to reconstitution of a piece of hydroid stem or planarian body after removal of apical or anterior regions, but Runnström and Hörstadius have interpreted it in terms of the two opposed concentration gradients.⁵ Basal halves include the most basal part of the presumptive ectoderm (*veg*₁, Fig. 145), the whole presumptive entoderm (*veg*₂, Fig. 145), and the primary mesenchyme. According to Hörstadius, they give rise to a variety of forms ranging from normal plutei, through various modifications of pluteus form and more or less ovoid larvae with gut proportionally too large and with abnormal, often excessive skeleton, to exogastrulae. The teratological forms resemble forms resulting from differential inhibition by various agents. In the forms which approach or attain normal proportions there is evidently considerable reconstitution of ectoderm. In apical halves the animal gradient is supposed to gain the upper hand, according to Hörstadius; one might expect, then, that in the basal halves the vegetal gradient would gain the upper hand, but it certainly does not in the forms that reconstitute ectoderm. These cases also seem to be similar to post-embryonic reconstitutions in the lower invertebrates. After isolation from the higher gradient-levels of the apical half more or less activation of the more apical levels of the basal half apparently increases scale of the ectoderm and raises gradient-levels so that apical regions can develop. This

⁵ Runnström, 1928a; Hörstadius, 1928a, b, 1935.

alteration appears essentially similar to the activation of levels of the hydroid stem or the planarian or annelid body after isolation to a condition making possible hydranth or head development.

On the other hand, removal of the higher levels of the primary gradient may result in earlier development and greater extent of the secondary gradient. The forms with too large gut and excess skeleton and the exogastrulae probably represent cases in which the secondary gradient has "gained the upper hand" to greater or less extent and partly inhibited or obliterated the primary gradient. This series of forms is interesting in comparison with the series of inhibited head forms in planarian reconstitution. There the inhibition of head development results from the activation following section at the posterior end of the piece. In the basal sea-urchin half it apparently results from activation and development of a secondary gradient in the basal region, but the effects on the primary gradient show interesting similarities in the two cases.

Reconstitution of isolated *veg*₁ and *veg*₂ (see Figs. 145, 147) has also been followed by Hörstadius (1935). The *veg*₁ ring represents the most basal third of presumptive ectoderm and may develop as an entirely ectodermal blastula but less extremely apical than the apical half; or it may reconstitute primary mesenchyme and entoderm, gastrulate, and sometimes approach normal pluteus form, but development is slow. Hörstadius accounts for the differences in development by the assumption of variation of the third cleavage plane from equatorial to subequatorial, so that *veg*₁ is more basal in some individuals than in others. There may be such variation, but the assumption is necessary only for the hypothesis of overlapping substance gradients. In terms of primary and secondary metabolic gradients the range of variation in form is accounted for by quantitative differences in metabolism and in degree of dominance and of basal activation, either characteristic of different eggs or resulting from differences in experimental procedure and environmental conditions.

Isolated *veg*₂ rings, though entirely entodermal in normal development, reconstitute ectoderm but do not develop apical tuft or stomodeum which are characteristic of the more apical ectodermal levels. Apparently the activation in the primary gradient following isolation is less than in *veg*₁, again a parallel to hydroid and planarian reconstitution. Here, again, the vegetal gradient does not "gain the upper hand," though it might be expected to do so, according to Hörstadius' interpretation of various other cases. However, these *veg*₂ rings develop relatively large entoderm and some become exogastrulae; but skeletal development is slight, probably

because ectodermal development does not proceed far. Reconstitution of ectoderm is difficult to account for in terms of the Runnström hypothesis, but in terms of a quantitative primary gradient it is again essentially similar to what happens in isolated pieces from the lower levels of hydroid stems and planarian bodies.

Isolation of the whole presumptive ectoderm (an_1 , an_2 , veg_1 , Fig. 145) results in forms either wholly ectodermal, with ciliated band surrounding an oral region, a stomodeum, but no oral lobe or arms and no skeleton (Fig. 164, *A*, *B*), or with small entoderm and rudimentary skeleton (Fig. 164, *C*, *D*). These isolated parts represent approximately three-fourths

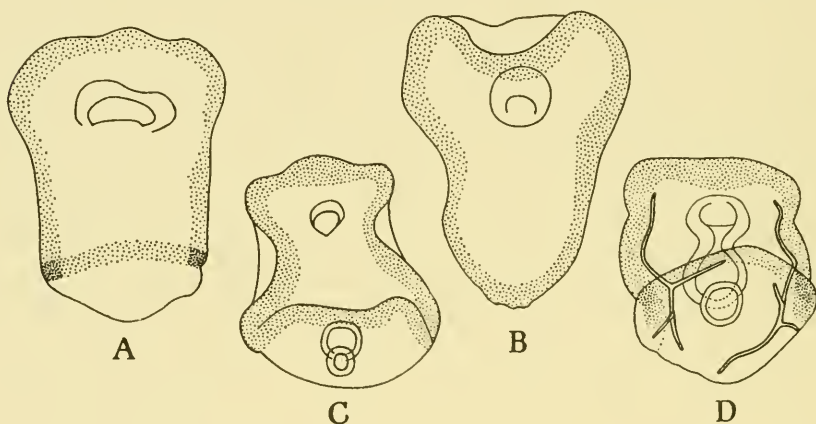


FIG. 164, *A-D*.—Range of forms resulting from reconstitution of whole presumptive ectoderm (an_1 , an_2 , veg_1) of *Paracentrotus*. *A*, *B*, with large scale of organization along primary gradient; *C*, *D*, with smaller scale of organization (after Hörstadius, 1935).

of the polar axis; consequently, concentration of vegetal substance should be considerable in their basal regions, but many of them develop no trace of entoderm. Moreover, veg_1 alone develops entoderm more frequently and may approach the normal pluteus form, as shown above. How is the very different development of the same axial level in the two cases to be accounted for? According to Hörstadius, there is lack of balance between the two gradients in the isolated whole ectoderm, and the animal gradient may suppress the vegetal, while in isolated veg_1 the two are more nearly balanced. In terms of metabolic gradients the ectodermal gradient and dominance may persist in the apical three-fourths because with good physiological and external conditions there is nothing to determine entodermal levels. With less vigorous animals or with slightly inhibiting con-

ditions—perhaps contact of the basal region with bottom of the container, resulting in a decrease in the oxygen supply—a small entodermal region may be determined. Primary and secondary gradients are more nearly balanced in isolated *veg*₁ than in isolated whole ectoderm; but in terms of primary and secondary activity gradients the balance results from the changes following isolation rather than from pre-existing concentration gradients. It was noted earlier that Hörstadius' interpretations often seem to be in terms of metabolism, rather than of concentrations, and to imply something very similar to dominance.

Apical halves with the ring *veg*₂ added gastrulate before forming mesenchyme but develop into plutei with relatively small entoderm, although only two-thirds of the presumptive ectoderm and the whole presumptive entoderm are present. Hörstadius accounts for the small size of entoderm by the fact that the presumptive entoderm also forms mesenchyme. Mesenchyme formation, however, occurs late, "at the end of invagination," when the size of the entoderm must be more or less determined; and it seems improbable that the small number of cells forming mesenchyme would decrease entodermal size very greatly. It is suggested as another interpretation that in this case *veg*₂, being brought into direct relation with more apical levels of the primary gradient, is partly ectodermized.

Whole ectoderms (*an*₁, *an*₂, *veg*₁) with four micromeres added basally differ markedly in development from whole ectoderms alone. They give rise to plutei, some scarcely distinguishable from controls, but most with entoderm somewhat too small. In this combination presumptive entoderm is absent, but entoderm of normal or almost normal proportions is reconstituted. Apparently the implanted micromeres bring about this entodermization of presumptive ectoderm, as they do when implanted elsewhere in ectodermal regions. Hörstadius holds that they increase the vegetal gradient, presumably by producing vegetal substance; von Ubisch, that they attract vegetal substance. There is also the possibility that, whether they are or are not specifically different from other cells in early stages, they initiate basal activation and the secondary gradient, which is apparently a factor in gastrulation and differentiation of entoderm. They may even be more effective in this way in consequence of isolation from their original relations to other cells.

Apical halves with *veg*₂ and micromeres added include all the presumptive entoderm but only two-thirds of the presumptive ectoderm. They give rise to plutei with somewhat too large entoderms and thus differ from apical halves plus *veg*₂, in which the entoderm is small. Here, again,

the micromeres apparently bring about increase in size of entoderm, whether by increased activation of the basal region or otherwise.

Other experiments show entodermization of presumptive ectoderm in somewhat different ways. A small group of cells derived from *an*₁, stained for identification and implanted in the basal region, may become part of the entoderm. Combination of the eight apical cells of the sixteen-cell stage (stained) by their basal surfaces with the median surface of a meridional half results in larvae in which stained cells of presumptive ectoderm form almost half of the entoderm (Hörstadius, 1928a, b; 1935). Here either vegetal substance presumably diffuses from the basal region of the meridional half into presumptive ectoderm of the apical half and entodermizes it or the activation in the basal region of the one induces the secondary gradient in presumptive ectoderm of the other, either dynamically or by diffusion.

The preceding experiments have been concerned with apicobasal pattern only. Certain others, bearing on the problem of ventrodorsality, are also of interest. According to earlier authors, 1/2 and 1/4 blastomeres reconstitute whole larvae; but certain defects, particularly in skeletons of larvae from isolated 1/2 blastomeres, were described by Plough (1927, 1929). Halves and quarters of the earlier cleavage stages of *Paracentrotus*, isolated in planes of first and second cleavages, may give normal or practically normal plutei—in fact, four almost completely normal plutei may develop from a single egg (Hörstadius und Wolsky, 1936). Orientation by staining of the surfaces of separation of these halves and quarters leads the authors to conclude that first and second cleavages may have any relation to the median plane, that ventral halves and quarters develop more rapidly than dorsal, and that the original ventrodorsality persists in ventral but is inverse in dorsal halves and quarters, the original dorsal side becoming ventral, the original ventral, dorsal. The more rapid development of ventral isolates is to be expected since the high region of the ventrodorsal gradient is ventral (pp. 134–40). Lindahl (1932) and Hörstadius have also suggested a higher metabolism ventrally than dorsally. Inversion of ventrodorsality in the dorsal half or quarter apparently represents activation of the low region of the ventrodorsal gradient in consequence of isolation; possibly inhibition by staining of the surface of separation may also be concerned in bringing about the inversion. Right and left halves, stained on the surfaces of separation, are usually defective on the stained side. Isolated meridional eighths of thirty-two-cell stages without micromeres reconstitute larvae approaching pluteus form more

or less closely, but usually with defective skeleton and incomplete development or absence of oral lobe and anal arms; they may attain practically complete bilateral symmetry of form.

GENERAL CONSIDERATIONS CONCERNING THE
SEA-URCHIN EXPERIMENTS

Reconstitution of a whole of small size from an isolated part involves decrease in scale of organization, that is, a decrease in scale of the gradient system. Development is usually slower in these reconstitutions than in controls, and, according to Tyler (1933), more energy is required to attain a particular developmental stage than in the normal whole. That the slower development and greater energy requirement of the isolated parts is not a matter of the time required for "regulation" is indicated by the fact that giants resulting from fusion of two fertilized eggs develop more rapidly than embryos from single eggs. The range of decrease in scale of organization is rather narrowly limited in the sea-urchin embryo; consequently, isolated parts frequently develop into forms differing in proportions of differentiations from higher and lower gradient-levels or partial forms in which scale of organization is larger than the isolated part.

The parallelism between disproportionate and apical partial forms in sea-urchin reconstitution and in postembryonic reconstitutions of hydroids and planarians and many annelids is striking, and decrease in scale of organization by inhibiting conditions has essentially similar effects in both. In fact, the isolations and transplantations of early sea-urchin development seem to show that the primary apicobasal patterns concerned in them and in the postembryonic reconstitutions of lower invertebrates are not fundamentally very different. The embryonic reconstitutions of the sea urchin bring to light little that differs essentially from the data of reconstitution of mature individuals.

It has already been pointed out that two opposed substance gradients may be associated with a single activity gradient and that the latter may be the effective factor in development (p. 241). The assumption of two opposed specifically different metabolisms coexisting in the same region, each tending to "suppress" the other but each supposed to persist unless completely suppressed, and appearing as required to account for experimental results, involves difficulties. Even if two opposed concentration gradients of substances are present, a single metabolic gradient may be expected as a resultant. Moreover, the evidence from other reconstitutions indicates that physiological activity, rate or intensity of metabolism,

is more nearly a primary factor in developmental pattern than specific substance differences.

Between the data of embryonic sea-urchin reconstitution and those indicating existence of a primary, and later of a secondary, activity gradient there is no conflict. Different substances may be present in apical and basal regions or be formed as development progresses; and as far as they are present, they are doubtless factors in differentiation of ectoderm and entoderm, but entodermization and ectodermization in consequence of isolation and combination of parts and of differential susceptibility to chemical and physical agents appear to be more directly related, at least as regards their initiation, to nonspecific differences in metabolic activity than to specific substances.

In the echinoids for which data are available the secondary basal region of activation becomes more effective as a dominant region and apparently as an inductor than the apical region. This accords with the fact that dye reduction and susceptibility suggest that it represents a more intense activation than any other part of the embryo at the stages concerned. Also, entodermization of presumptive ectoderm seems to occur more frequently in Hörstadius' experiments than ectodermization of presumptive entoderm. Probably future experiment will show differences in different echinoid species in activation of the basal region. No data of differential dye reduction have been obtained for *Arbacia punctulata*, and the capacities of the micromeres as inductors are not known, but the characteristics of gastrulation suggest that basal activation may be less intense in that species than in some others. It seems to be less in the asteroid *Patiria*, preceding invagination, than in echinoids (p. 134).

RECONSTITUTION IN INSECT DEVELOPMENT

Investigation of reconstitucional potencies in eggs and embryos of most arthropods is, to some extent, limited by the character of the chorion and the impossibility of removing the earlier stages from the egg envelope. However, by means of ligature at different levels, localized cautery, and ultra-violet radiation it has been possible to obtain some information concerning potencies of early developmental stages and the progress of physiological differentiation. Earlier experiments on chrysomelid beetles indicated absence of reconstitution at the developmental stages concerned (Hegner, 1908, 1911). Results of ligaturing and microcautery of early stages of fly development also indicated that at the earliest stages available after eggs were laid reconstitution did not take place (Reith, 1925;

Pauli, 1927). It appears from recent work that loss of a certain amount of oöplasm in consequence of puncture very soon after egg-laying does not prevent normal development in *Drosophila melanogaster*, but with similar loss at somewhat later stages abnormal imagines may result.⁶

According to the conclusions reached by Seidel from ligaturing, local cautery, and ultra-violet radiation of developmental stages of the libellulid *Platynemis pennipes*, the developmental pattern of this species differs from any pattern known in other animal groups.⁷ The embryonic primordium of this species becomes visible first as two lateral areas in which nuclei are more numerous than elsewhere (Fig. 165, *A*). These two areas come together on the ventral surface; the cephalic lobes appear anteriorly as still more densely nucleated areas and at the posterior end of the primordium the level at which inversion of the embryo into the yolk begins is also indicated by denser nucleation (Fig. 165, *B*). Seidel maintains that the region between the level at which the posterior end of the embryonic primordium normally forms and the posterior end of the egg is an initiative or formative center (*Bildungszentrum*) and that a substance essential to embryo formation passes anteriorly from it. Complete separation by ligature or killing of this region, during stages after cleavage nuclei have reached the egg surface and before blastoderm formation, prevents embryo formation but does not prevent further cell proliferation and formation of a cellular layer without definite pattern over the whole surface anterior to the level of injury, a nonembryonic blastoderm (Fig. 165, *C*). With complete separation by ligature at more anterior levels, embryo formation occurs only posterior to the ligature and a uniform anaxiate cell layer anteriorly (Fig. 165, *D*, *E*); but with incomplete ligature that does not destroy protoplasmic continuity, more or less development may occur both anterior and posterior to the ligature, as in Figure 165, *F*, in which a large head region develops anterior to, and an elongated embryo posterior to, the ligature. As development progresses, separation of the posterior region has progressively less effect on development anterior to it; in late blastoderm stages separation or killing of posterior parts has no inhibiting effect anteriorly. Incomplete ligature which does not destroy protoplasmic continuity does not prevent or inhibit development anteriorly even in early stages, because, as Seidel maintains, the substance from the *Bildungszentrum* can still pass.

As far as embryonic development is concerned, the primary effect of the

⁶ Howland and G. P. Child, 1935; Howland and Sonnenblick, 1936.

⁷ Seidel, 1926, 1928, 1929a, b, 1931, 1934, 1935.

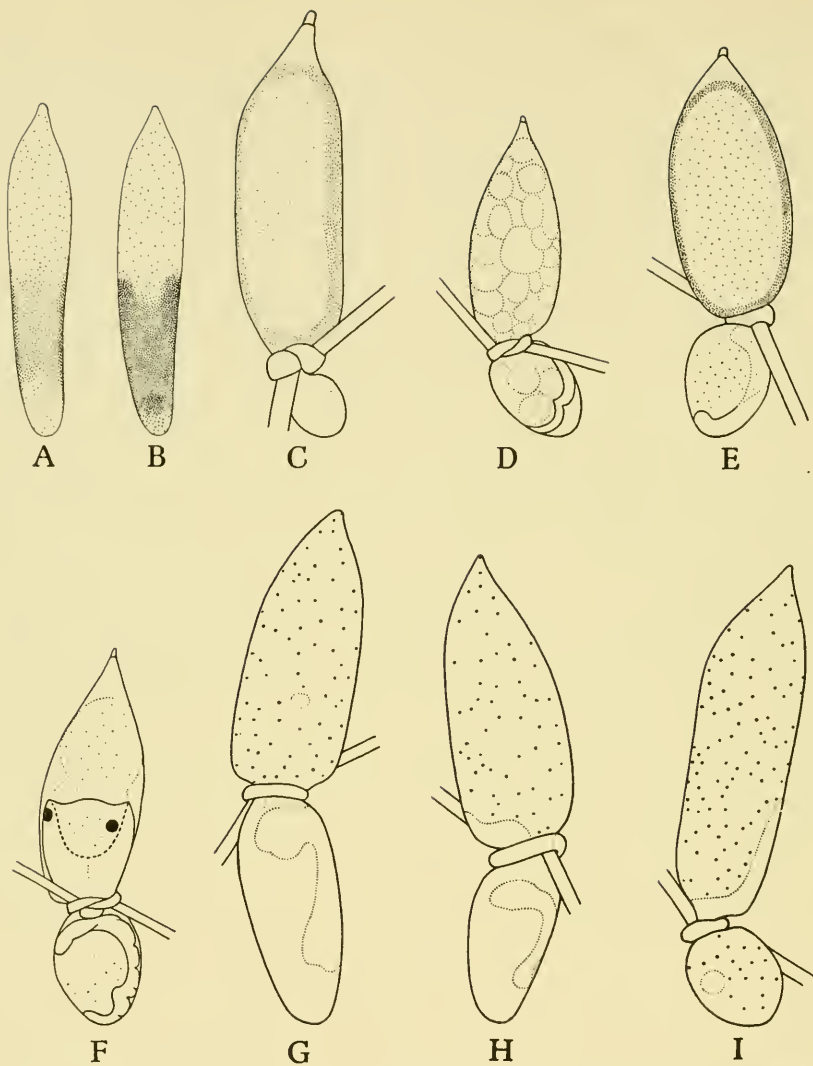


FIG. 165, A-I.—Normal embryonic primordium of the libellulid insect *Platycnemis pennipes* and results of ligaturing in early stages. A, two lateral areas more densely nucleated than other areas in early stage of normal development; B, later stage with cephalic lobes and region of beginning inversion of posterior end of primordium indicated by denser nucleation; C, result of complete separation of posterior region by ligature in early stages, inhibition of embryo formation; D, E, complete ligature at more anterior levels, embryo formation only posterior to ligature; F, incomplete ligature, head formation anterior, and embryo posterior to ligature; G, incomplete ligature in blastoderm stage anterior to differentiation center, embryo only posterior to ligature; H, incomplete ligature at blastoderm stage in differentiation center, embryos both anterior and posterior to ligature; I, ligature posterior to differentiation center, embryo only anterior to ligature (after Seidel, 1929b, 1934).

Bildungszentrum at the posterior end of the egg is to determine a "differentiation center," corresponding in position to the prothoracic segment. The aggregation and more rapid division of nuclei begins there, and development proceeds both anteriorly and posteriorly from this region. Seidel (1934) regards the differentiation center as in some way an organizer, but not by means of a substance, since incomplete ligatures block its action. With incomplete ligature anterior to this center in early blastoderm stages no embryo formation occurs anterior to the ligature (Fig. 165, *G*); but with ligature in the differentiation center embryo formation may occur both anterior and posterior to it (Fig. 165, *H*), and with ligature posterior to the center an embryo forms only anterior to the ligature (Fig. 165, *I*).

With other insects as material other investigators have found that posterior ligature or cautery in very early stages prevents embryo formation but at later stages may be followed by normal or partial development, according to species.⁸ As regards the differentiation center, however, there is not complete agreement. According to Reith, the head region is the differentiation center in *Camponotus*, but the evidence concerning the beetle *Sitona* is inconclusive. He suggests that the differentiation center is the region of "greatest physiological activity." The embryo of the honeybee, which occupies the whole length of the egg, shows very considerable reconstitucional potency, even 24 hours after laying. Dwarf complete embryos may develop posterior to a ligature separating the anterior fifth of the egg, but there is usually no development anterior to the ligature. With ligatures further posterior there may be partial development both anterior and posterior. Seidel's hypothesis of a prothoracic differentiation center is regarded as supported by these experiments (Schnetter, 1934*a*, *b*; 1936).

Extensive duplications of any or all parts have been produced in an orthopteran, *Tachycines asynamorus*, by local injuries and by separation, by glass needles, of parts of the small embryonic primordium situated at the extreme posterior end of the egg; and presence of a thoracic differentiation center is inferred, chiefly, it appears, from a single case of duplication (Krause, 1934). In a later paper Krause (1938) finds the high point in cleavage and sequence of differentiation at the level of the cephalic lobes; evidence for a differentiation center in this orthopteran appears only in the space-time order of differentiation posterior to the head.

Early developmental stages of *Drosophila* subjected to certain ranges

⁸ Reith, 1931, an ant, *Camponotus ligniperda*; 1935, a beetle, *Sitona lineata*.

of intensity and duration of ultra-violet radiation show no defects in embryonic or larval stages, but teratological modifications and defects appear in the imago (Geigy, 1931). Radiation of different early stages shows, according to this author, a sensitization progressing "in the craniocaudal or, better, thoraco-abdominal" direction, followed by a densensitization progressing in the same direction. Modifications of the imaginal head were not obtained in any case. These experimental results lead the author to accept the hypothesis of a prothoracic differentiation center.

In the lepidopteran *Ephesia* posterior cautery does not prevent axiate development, though it may produce defects of posterior regions. Cell formation, as nuclei reach the egg surface, progresses anteriorly and posteriorly from the differentiation center (Maschlanka, 1938).

Experiments with a beetle, *Bruchus quadrimaculatus*, show prevention of axiate development by posterior constriction or cautery in early stages, with decreasing effect as development progresses. The differentiation center is maxillar-prothoracic (Brauer and Taylor, 1936). In further experiments with the same species on developmental modification in relation to susceptibility to KCN, differential inhibitions of development have been obtained. With certain periods of exposure to cyanide in early stages, the median ventral maxillar-prothoracic region shows the greatest injury with decrease anteriorly, posteriorly, and laterally from the anteroventral position of this region (Fig. 166, A-C). With inhibition of development, various degrees of embryonic duplication may result, beginning at the level of the differentiation center and extending anteriorly and posteriorly from it, according to degree and extent of the differential inhibition, occasionally resulting in complete duplication (Fig. 166, D, E). In these cases the less susceptible lateral regions are physiologically isolated by inhibition of the median region and with recovery develop independently. At stages when the embryonic plate is elongating, inhibition of the elongation is the most conspicuous feature of modification by cyanide (Brauer, 1938).

In embryos of the beetle *Tenebrio molitor* after posterior cautery in early stages, heads, or even anterior parts of heads, may develop in complete absence of all other parts (Ewert, 1937); the thoracic differentiation center is not evident.

All workers in this field agree, however, in finding that major reconstitutions occur only in early developmental stages, if at all; but, according to Seidel's account, the pattern of early insect development is unique. The hypothesis of a differentiation center activated or determined by another center in the posterior region of the egg raises certain questions. First, as

regards the *Bildungszentrum*, how does a substance diffusing from this center activate or determine an axiate pattern with region of greatest developmental activity near the anterior end of the future embryo, that is, farthest away from the source of the substance? A complete ligature in

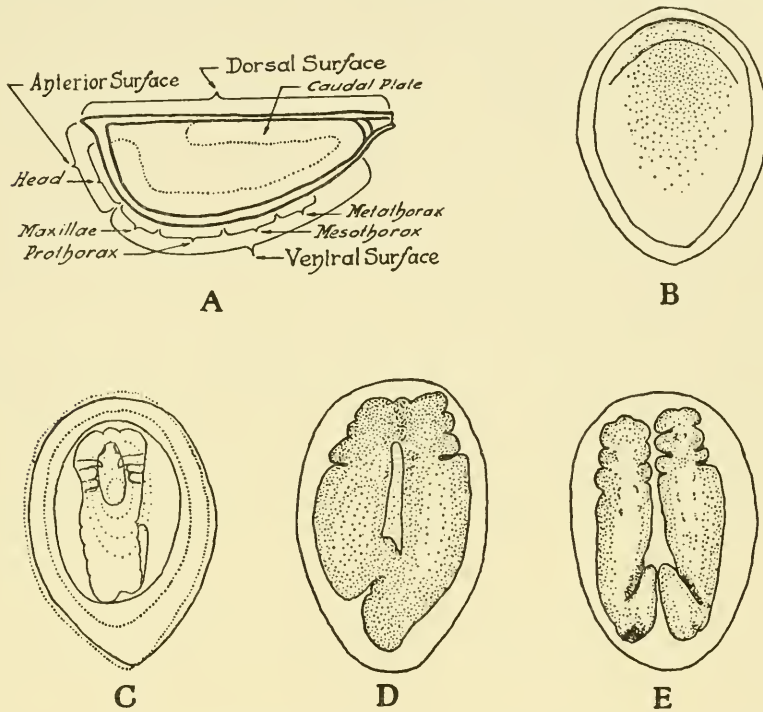


FIG. 166, A-E.—Differential modification of development by KCN in the coleopteran *Bruchus quadrimaculatus*. A, diagram of a median longitudinal section showing surfaces and presumptive embryonic regions; B, ventral view of early stage, indicating by density of shading the differential susceptibility to KCN, decreasing from the median maxillar-prothoracic region posteriorly and laterally; C, embryo with maxillar-prothoracic defect resulting from KCN treatment in early stage (unbroken lines), superimposed on early embryo fixed 2 hours after KCN treatment (dotted lines) with susceptibility, as indicated by injury, decreasing from inner to outer elliptical lines; D, later embryo, showing partial duplication by KCN treatment, complete in thoracic region and extending anteriorly into the head and posteriorly through the thorax; E, complete duplication by cyanide treatment (from Brauer, 1938).

the posterior region is supposed to block the movement of this substance; but it also breaks the egg cortex, and a wound stimulus must result and extend anteriorly from what is essentially a level of section. This may obliterate more or less completely the anteroposterior gradient in the embryonic region. With incomplete ligature continuity of the cortex is not

interrupted, though in the ligature experiments in relation to the differentiation center at somewhat later stages the susceptibility of the cortex may have increased to such an extent that incomplete ligature is sufficient to produce a stimulus decreasing or obliterating the gradient of more anterior regions. The effect of cautery should be essentially similar to that of section of the cortex by ligature. The insect egg is apparently highly sensitive. Seidel observed contraction of the yolk about cauterized regions, and Reith (1925) saw a wavelike contraction (*Zucken*) pass over the egg of *Musca* when it was cauterized locally. The assumption that a part of the insect egg can be pinched off by ligature or killed by burning without activation or excitation extending to other parts seems, at present, unwarranted, in view of the data on postembryonic reconstitutions and some other embryonic reconstitutions. With complete obliteration of the anteroposterior gradient by an opposed gradient the embryonic region must become apolar, as it actually does after complete ligature and cautery in early stages. It gives rise to a nonembryonic blastoderm without differentiation. In early stages the activation or excitation resulting from posterior ligature or cautery may be sufficient to obliterate the whole embryonic gradient, or its effect may be partial and involve only abdominal, or abdominal and thoracic, regions (Brauer and Taylor, 1936). In *Tenebrio* a head, or only the anterior part of a head, may develop after posterior cautery (Ewert, 1937). Later, after the anteroposterior gradient and determination along its course have progressed farther and become more stable, posterior ligature or cautery or ligature has little or no effect. It may also be questioned whether inhibition of development anterior to a ligature just anterior to the differentiation center is due to isolation from the center or to obliteration of the gradient in the anterior region by the opposed gradient resulting even from incomplete ligature in the more susceptible anterior region. In short, the experimental data suggest that inhibition of development anterior to a region of ligature or cautery may be due, not to isolation from a center, but to obliteration of an anteroposterior gradient by an opposed activation or stimulus resulting from the injury. Absence of development posterior to a ligature in early stages is doubtless due to isolation from a dominant region. These suggestions are in line with results of postembryonic reconstitutions in lower invertebrates. Section a short distance proximal to a hydranth primordium in early stages may inhibit it completely, and posterior section within a certain distance of the anterior end of a piece inhibits planarian head development. No *Bildungszentrum* is involved in these cases. Apparently there is

not complete agreement as regards presence of a *Bildungszentrum* in all insects investigated. According to the gradient concept, it is to be expected that ligature and cautery will be more effective in some species than in others in obliterating the polar gradient at more anterior levels. This is the case with posterior section in different planarian species.

As regards the prothoracic or maxillar-prothoracic differentiation center and its significance, or even its presence in certain forms, there seems also to be difference of opinion. If heads or anterior head regions can develop in its absence, it does not appear to be essential unless it is present in the cortical cytoplasm before nuclei reach the cortex, as some believe it to be. The question whether it possesses inductive capacity or is merely a region in which cell formation is more rapid and susceptibility greater than elsewhere seems still to be open. Certainly prothoracic regions can be reconstituted in other regions of the egg than the original presumptive prothoracic region. If there is a prothoracic region of primary dominance, the head is apparently secondary in origin; that is a unique polar pattern. However, axiate pattern may be present in the cortex before nuclei reach it; the head region may be a primary feature of that pattern; and the prothoracic differentiation center a secondary development associated with nucleation of the cortex. Early insect development remains an extremely interesting problem.

RECONSTITUTION IN EARLY FISH DEVELOPMENT

Development of isolated blastomeres of the holoblastic lamprey egg indicates presence of a dorsal inductor region more or less similar to that of amphibians.⁹ Early embryonic stages of the meroblastic fish eggs show high capacity for reconstitution. The cleaving blastoderm of *Raja*, separated by section into fourths, may give rise to several embryonic primordia, even though the separated parts gradually unite again to a single disk (Eismond, 1910). However, a differential appears to be present in the blastoderm, for the primordia develop from median and lateral regions of one half, none developing from the other half; and retarded development and early degeneration of the lateral primordia after reunion of parts suggests that their further development is inhibited by dominance of more nearly median primordia, which probably represent higher gradient levels of the blastoderm.

In teleosts normal embryos may develop from parts of the germinal disk, from $1/2$ blastomeres, from two $1/4$ blastomeres, from six $1/8$, and

⁹ Bataillon, 1900a, b; Montalenti e Maccagno, 1935.

from twelve 1/16 blastomeres in various combinations, and even after removal of the whole embryonic area up to a certain stage preceding gastrulation.¹⁰ Removal by localized puncture or cautery of parts of the embryonic shield after its formation indicates, in general, progressive stabilization of course of development; but apparently normal, or only quantitatively defective, development is possible after removal of small amounts, even of axial material. Injury of the posterior end of the embryonic region in earlier stages may completely inhibit embryo formation, perhaps by obliteration of the polar gradient. In later stages it results in absence of posterior parts still to be formed at the stage of injury. Recent experiments, using the method of implantation of isolated parts of the blastoderm into the yolk sacs of other embryos, show similar development of all sectors of the blastoderm in pregastrula stages (Mangold, 1931*c*; W. Luther, 1936*a, b*). Sectors including the marginal region, when thus implanted, are capable of developing neural tube, notochord, muscle segments, intestine, auditory vesicles, eye lenses, blood vessels, blood corpuscles, kidney canals, and liver tissue. Reconstitution of the extraembryonic sectors apparently represents an approach to development of a new embryonic primordium. Further experiments on removals and transplantations of parts of the blastoderm of the trout also show high capacity for reconstitution in earlier stages (W. Luther, 1937*a, b*). Combination of two extraembryonic halves in the blastula stage by replacing the embryonic half by an extraembryonic half results in reconstitution of complete, or almost complete, embryos in 90 per cent of the cases. In early gastrula stages an embryo develops after removal of a lateral half of the embryonic sector, either by movement into its place of material still farther lateral or when it is replaced by a piece from the extraembryonic half. Reconstitution follows removal of either lateral or median part of the embryonic region. In the early gastrula the region capable of development extends about 45° right and left of the longitudinal embryonic axis, but reconstitution sometimes occurs after replacement of somewhat more than 90° of the embryonic sector by an extraembryonic piece. With progress of gastrulation the reconstitutive capacity of extraembryonic regions of the blastoderm gradually disappears, first in the median region opposite the embryonic sector and from this region progressively on both sides. In the later gastrula capacity for development corresponds approximately to the prospective organ regions.

Luther's interpretation of these data is that capacity for development

¹⁰ Morgan, 1895*a*; Kopsch, 1896, 1904; Sumner, 1903; W. H. Lewis, 1912*a, b*; Hoadley, 1928.

depends on level of metabolic activity, that up to the gastrula stage all parts of the blastoderm have a sufficiently high level to reconstitute axial embryonic organs, but this gradually decreases from the beginning of gastrulation. There is, however, a gradient of decreasing activity laterally and around the blastoderm from the region of the longitudinal axis. In earlier stages lateral extraembryonic parts are active enough to replace median embryonic parts, but this capacity is gradually limited to the embryonic quadrant and finally to the organ regions. In connection with this interpretation the possibility may also be noted that extraembryonic regions may undergo activation when isolated, or by induction when implanted in contact with higher gradient-levels. The data on differential susceptibility are in line with Luther's interpretation in that they show that the embryonic quadrant attains a higher susceptibility than other regions in early stages.

Certain experimental data suggest presence of a differential and some degree of dominance, even before formation of the embryonic shield. Under inhibiting conditions more than one embryo may develop from the teleost blastoderm.¹¹ Evidently reconstitution of an embryonic region, inductor, and axis quite independently of the original is possible in teleosts, and the fact that normally only one embryo forms suggests that the presumptive embryonic region or sector attains some degree of dominance over other parts. However, as far as developmental capacities are concerned, the regional differences in early stages are apparently nonspecific quantitative differences in activity in a gradient pattern.

Pieces of *Fundulus* germ ring 90° and 180° from the dorsal lip of gastrulae at any stage, when implanted in extraembryonic regions or in the pericardium, form only epidermis, blood, and chromatophores but, when implanted in the embryonic shield, may develop almost any embryonic part, according to position, or sometimes parts not in accord either with position or with presumptive fate. Some influence, a dominance of some sort by the embryonic shield, is necessary for differentiation of these pieces of germ ring into definite embryonic organs. No evidence of gradation of developmental potencies around the germ ring has been found in this material (Oppenheimer, 1938).

CERTAIN RECONSTITUTIONS OF EARLY AMPHIBIAN DEVELOPMENT

When one blastomere of the two-cell stage is killed or injured so that it cannot develop, but is left in position, the other may develop as a half-embryo (Roux, 1888; A. Brachet, 1927); but, according to Roux, the half-

¹¹ Stockard, 1921; Hinrichs, 1925; see also F. Schmitt, 1901, 1902.

embryo may later become more or less a whole, either by reconstitution or, in case the other blastomere was not completely killed, by spread of nuclei into it and by cell formation from its cytoplasm (postgeneration). This apparent reconstitution of a half to a whole was the subject of much controversy. Combining killing of one blastomere with maintenance in inverted position, it was found that the living blastomere might develop as a whole, supposedly in consequence of a more complete isolation of the living from the killed blastomere through the rearrangement of materials by gravity (Morgan, 1895*b*). The blastomeres are too fragile to permit direct separation, but they may be separated by gradual ligation, or one may be removed by suction. With these procedures the $1/2$ blastomere may develop as a whole, or with incomplete separation by ligation various degrees of twinning may result.¹²

Partial separation of undivided eggs by ligation shortly after fertilization leaves one of the two parts without a nucleus. The nucleated portion develops, the nonnucleated does not; but if the ligation does not completely separate the two parts, a cleavage nucleus may sooner or later pass through the constriction into the nonnucleated part, and this may then begin to develop. Such cases may result in partial or complete twinning, the originally nucleated portion being in advance of the other. Moreover, it was found that complete separation of the first two blastomeres by ligation or otherwise gave different results according to relation of plane of first cleavage to the median plane. When the first cleavage plane coincides with the median plane, both right and left half are able to develop as wholes; but when dorsal and ventral halves are separated, the dorsal develops as a whole, but the ventral forms only a rounded mass, showing some development of germ layers but without axial organs. The dorsal inductor region is responsible for this difference in development. With median first cleavage both blastomeres contain half of this region; and, since the isolated half is able to induce whole development, each may develop as whole. When the first cleavage is frontal, the inductor is wholly in the dorsal blastomere. Apparently the same differences result from ligation of the undivided egg in different planes.¹³ These differences in development are regarded as indicating early localization of the dorsal inductor

¹² Endres, 1895; Herlitzka, 1896; Spemann, 1901*b*, 1902, 1903*b*; McClendon, 1910; Rand, 1925; G. A. Schmidt, 1930, 1933.

¹³ Spemann, 1901*b*, 1903*b*, 1914, 1928, 1936, pp. 16-18; Fankhauser, 1930*a*, *b*; also Streett, 1940, "Experiments on the organization of the unsegmented egg of *Triturus pyrrhogaster*," *Jour. Exp. Zool.*, 85.

region. Separation of halves by ligature in later cleavage, blastula, or even early gastrula stages shows much the same differences, according to plane of separation. After median separation the inner sides of the two individuals are, in general, increasingly less developed than the outer sides, the later the stage of separation. The left member of the pair has normal situs viscerum, but in the right member situs inversus is not rare.¹⁴ Contrary to the earlier conclusions of A. Brachet from experiments with local cautery, ligature and removal of parts showed that early anuran embryos have essentially the same capacity for reconstitution as the urodele (Vogt und Bruns, 1930; G. A. Schmidt, 1933).

These reconstitutions in the early stages of amphibian development indicate that the dorsal inductor region is in some way localized but that, within it, relations of dominance and subordination and capacity for reconstitution are present, and the symmetry pattern, if definitely present in these stages, may undergo extensive reconstitution. However, the fact that new dorsal inductors may originate in any part in inverted and partly inverted eggs (pp. 428-30) indicates that determination of the inductor region in early stages has probably not proceeded very far in specific differentiation.

RECONSTITUTIONS IN THE AMPHIBIAN GASTRULA AND LATER STAGES

The discussion of amphibian inductors in the preceding chapter was largely concerned with reconstitution in consequence of altered relations to an inductor. It was shown there that an inductor may determine the course of development of other parts but that its own development may be largely or wholly independent of them and may depend on relations of dominance and subordination within the inductor region itself. When a region, field, or organ system has attained a certain stage of determination, chemodifferentiation, or physiological stability, reconstitution of a different region, field, or organ system from it does not occur under the usual experimental conditions, though we do not know that it might not occur under other conditions. In amphibian development progressive limitation or restriction of developmental potencies under known experimental conditions becomes evident in gastrula and later stages, but extensive reconstitution is still possible within various organ primordia and fields when parts are removed. Some organ systems, like legs and tails of urodeles, are capable of regeneration even in the adult. Reconstitution in an

¹⁴ Spemann und Falkenberg, 1919; Ruud und Spemann, 1922.

organ field is not necessarily limited to the region in which the organ actually develops but may take place in other parts of the field. Examples of this in the limb field, the optic field, and some others and their apparent relations to gradient patterns were considered in chapter xi.

An extensive investigation of the capacity for development of isolated parts of urodele and anuran gastrulae explanted in modified Ringer solution has shown that entoderm and parts of the mesoderm are capable of advanced differentiation, even in small explants, but largely according to prospective significance; that is, their development may involve more or less reconstitution but is not greatly altered by isolation and explantation. Parts of the dorsal inductor region similarly explanted show more extensive reconstitution of parts beyond their prospective significance. Lateral parts become bilateral; neural tubes and even epidermis may develop from these pieces. Explanted ectodermal pieces show no capacity for any definite pattern of development. They form neural tissue only in relation to an inductor. Also, gastrula ectoderm cultured for a time as explant and then implanted in relation to an inductor shows decreasing reactivity, the more advanced the stage from which it originates.¹⁵ As regards the general pattern of amphibian development, these experiments do not essentially alter earlier conclusions. They confirm Holtfreter in his belief in a mosaic of inductors in the dorsal inductor region, but this seems still to be a matter of opinion rather than a necessary conclusion from these or other experiments. The data show great differences in capacity for independent development; but the question how far these differences depend on degree of determination of parts, as Holtfreter believes, rather than on susceptibility to isolation and explantation is perhaps still open. It seems possible that entodermal regions, for example, may be no more determined than other parts in the sense of having attained a higher degree of specificity before isolation but that, because of their low metabolism at the time of isolation, they are not very susceptible to the altered conditions and continue development more or less according to the pattern of which they were originally a part. Parts of the dorsal inductor region are much more susceptible to isolation and show extensive reconstitution, even giving rise to ectodermal parts. Explanted ectodermal pieces apparently never attain a sufficiently high metabolic level to permit neural development.

A very considerable capacity for reconstitution appears in various organ primordia of later embryonic stages; for example, a complete limb may develop from a part of a limb bud or prospective limb region. In this

¹⁵ Holtfreter, 1938*a*, *b*, *c*, and literature cited in these papers.

connection the eye is of particular interest. In the early gastrula of *Triton* the whole presumptive epidermis can give rise to eyes, under influence of the dorsal inductor; but eye potency decreases from apical to basal regions, that is, down the primary gradient.¹⁶ After the neurula stage only the optic primordium gives rise to eye, but the capacity of the optic vesicle and optic cup for reconstitution of a whole eye from parts homoplastically transplanted to other embryonic regions supposedly without eye-determining factors—for example, the ear region—is great. Any part can reconstitute any part: even small pieces from any part of the tapetum can become whole eyes. In general, capacity for organization increases with size of the transplanted piece. The larger the piece, the more evident is the heteropolar pattern. Extremely small fragments usually give rise only to the pigment layer, but in larger pieces the retina may be massive.¹⁷ The orderly character of development in the transplanted pieces and the relation between organization and size of piece have led Dragomirow to regard the optic vesicle as a gradient system in which the retinal region is dominant. The pigment layer represents development from lower gradient-levels. He finds these embryonic optic reconstitutions closely analogous to reconstitutions in hydroids and planarians. Pieces of iris of urodele larvae of various ages implanted in the orbit after removal of eye or in the abdominal cavity may reconstitute retina; and in the orbit, nerve fibers (Monroy, 1939).

In marked contrast to the high reconstitucional capacity of the optic vesicle and various other parts in later embryonic stages is the failure of the early tail bud to reconstitute parts removed,¹⁸ although a high capacity for regeneration is characteristic of later stages of tail development. Two possible factors of this failure may be pointed out. For reconstitution presence of spatial pattern and reaction to removal of a part are necessary: a mass of cells all alike cannot reconstitute anything if part of the mass is removed; also, if level of activity is so high that removal of a part does not bring about activation, there is no reconstitution, and the remaining part continues as before. The early tail bud is evidently a mass of actively growing cells, perhaps with a slight radial gradient, like other buds, but probably with so little differential and dominance and so high a level of activity that removal of a part leaves the remaining part practi-

¹⁶ See Mangold, 1931a, for literature.

¹⁷ Dragomirow, 1932, 1933, 1935, and earlier literature cited in these papers. His experiments include three species of *Triton*, *Amblystoma mexicanum*, *Pelobates fuscus*, and *Bombinator igneus*.

¹⁸ Schaxel, 1922; Vogt, 1931; Svetlov, 1934.

cally unaltered. In later stages, when gradient pattern has developed, further removal of a part is followed by activation and increased growth near the cut surface, and regeneration results.

Some parts of the amphibian and other embryos, isolated after a certain stage under proper conditions, are capable of independent or self-differentiation, that is, they are able to continue for a time their original course of development. It is commonly assumed that such parts also differentiate independently when parts of the intact organism and that, in so far as parts attain this condition, the organism becomes a "mosaic" of independent parts. This conclusion does not seem entirely justified. Even though differentiation is independent after isolation, it may not have been before. In other words, self-differentiation of an isolated part may represent more than it accomplishes independently without isolation. The fact that it can differentiate independently is not proof that it does so in the intact organism.

RECONSTITUTION AND PATTERN IN THE AVIAN EMBRYO

Morphological and material aspects of developmental pattern in the chick have been subjects of investigation by various methods and of considerable controversy for many years. Local injury—mechanical, electrolytic, or by radiation—has been extensively used in attempts to throw light on the problem, and modifications resulting from subsection of embryonic stages to various chemical and physical agents have been described and analyzed. In recent years data on differential susceptibility and differential dye reduction, transplantations of parts to other embryonic regions or to the chorio-allantois, as a supposedly neutral site outside the embryo proper, and explantations to plasma clots with embryonic extract have given information concerning some of the physiological features of pattern and concerning regional and chronological differences in developmental capacity or potency under certain experimental conditions and their relations to the general pattern. Some of the evidence from these experiments is briefly reviewed.

Cell migrations are very slight during the first 10 hours of incubation, and extensive reconstitution is possible at these stages. Lesions produced electrolytically or by ultra-violet radiation in the median posterior region of the blastoderm result in various anomalies, among which are more or less complete embryonic duplications and supplementary heads (Twisselmann, 1938). The duplications are regarded as determined by the effect of experimental procedure on the inductor—inhibition or killing of the

median parts—with reconstitution of more lateral regions. This author also holds that his data support Dalcq's view that the inductor differences along the polar axis are quantitative, not qualitative.

Some of the earlier experiments on chorio-allantoic grafting were more directly concerned with questions of the degree of organization or determination attained at particular stages than with spatial pattern and led to the conclusion that progressive organization is arrested in a part by isolation; consequently, the differentiation of the isolated part is a measure of the degree of organization present at the time of isolation (Hoadley, 1926*a*, *b*; 1927). If this is true, it follows that early isolation of parts should give less differentiation than isolation at later stages and that reconstitution should not take place, but other experiments show extensive reconstitution.

Transplantations of parts of the unincubated blastoderm show extensive differentiation only when they include the intact posterior median quadrant, the region from which the primitive streak later develops. This quadrant alone, although much smaller than the anterior three-fourths, gives rise to almost all the tissues of the anterior embryonic region and shows as extensive differentiation as posterior halves or whole blastoderms or the node-level of whole blastoderms of definitive primitive-streak stage (Butler, 1935). This posterior quadrant is the most susceptible region of the early blastoderm (p. 162). According to Butler, pieces lacking this posterior region do not develop central nervous tissue and usually give rise only to gut, smooth muscle, heart, liver, and skin tissues. Pieces including part of this posterior region show higher frequency of graft development than anterior pieces, and transverse fourths from different levels show a posteroanterior gradient of decreasing frequency of development. Fourths from the posterior half show much less development than the whole half or the median quadrant; and longitudinal fourths show little differentiation, giving only gut, smooth muscle, and heart, probably, as Butler suggests, because of interference with the cell movements concerned in development of the primitive streak.

In very early streak stages anterior, purely ectodermal regions, explanted to plasma clots, may form neural tubes; pieces anterolateral to the streak form chiefly heart in stages before the groove appears, after that, heart and neural plate; and the region of the streak gives rise in pregroove stages only to erythroblasts, in groove stages to heart and erythroblasts (Rudnick, 1938*b*). These results differ widely from those obtained by Butler with chorio-allantoic grafting of pieces from unincubated blastoderms.

The earlier results of chorio-allantoic grafting with definitive primitive-streak, head-process, and somite stages seemed to indicate that only pieces from the node region were capable of extensive development and that this region could give rise to all parts posterior to its level as far as, and including, mesonephros; but, as the physiological condition indicated by the node moved posteriorly, its development was progressively restricted to more posterior parts; in general, it did not give rise to parts characteristic of levels anterior to its position (T. E. Hunt, 1931, 1932). Further experiments, however, modified this view. Posterior parts of advanced primitive-streak stages, containing no more than the posterior two-thirds of the streak, cultivated *in vitro*, are able to continue differentiation (Waddington, 1935a). Dalton (1935) also found presence of the node region unessential for development of axial structures and regarded the technique of grafting as an important factor in results obtained. Forebrain develops in chorio-allantoic grafts from the head-process stage, even from pieces entirely anterior to the head-process and without notochord (Stein, 1933). Heteroplastic grafts between duck and chick of parts of the primitive streak without the node may show more or less reconstitution and may also act as inductors (Waddington and Schmidt, 1933).

Reconstitution of any part and even of the whole primitive streak after removal occurs in blastoderms explanted to plasma-chick embryo extract after some 20 hours of incubation with development of normal, or almost normal, embryos (Waddington, 1932). The cell movements are regarded by Waddington as the chief factors in this reconstitution, but both the cell movements and the development of normal embryos in these experiments indicate presence and effectiveness of a physiological pattern in relation to which fates of parts concerned in reconstitution are altered.

Development of ectoderm, mesoderm, and entoderm in small pieces from the different regions of the head-process blastoderm grafted on the chorio-allantois is shown in the maps of Figures 167, 168, and 169 (Rawles, 1936). Evidently considerable reconstitution takes place in many of the pieces, for various organ tissues develop in parts which, so far as known, do not normally give rise to them. Many of the potency fields are more extensive than the regions of actual differentiation in normal development, and Rawles finds that in each field developmental potencies for the part concerned decrease peripherally from a center. On the other hand, each such field is a more or less restricted region or level of the blastoderm. Some organs (heart, liver) develop only in lateral areas; others (notochord, suprarenal, spleen) only in median areas; and still others in both median

and lateral areas but in higher percentage in median (brain parts, eye, ear, mesonephros). The highest frequency of graft development is in median pieces including head process and node, but pieces from the left side show a higher frequency than those from the right. Development of

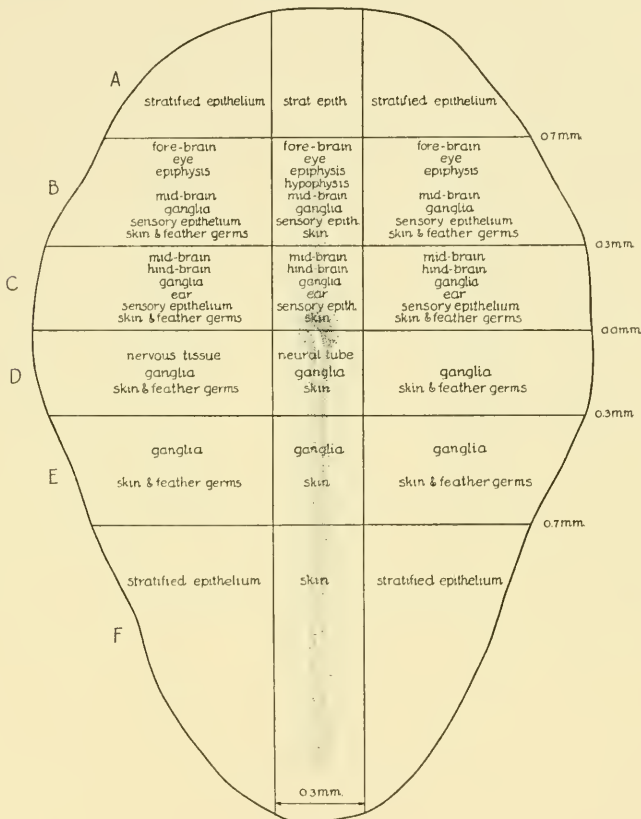


FIG. 167.—Map showing regional distribution of development of ectodermal structures in chorio-allantoic grafts of the head-process blastoderm of chick; distances from the primitive pit indicated in millimeters are average measurements (from Rawles, 1936; prepared by Willier and Rawles).

the pieces indicates an anteroposterior and mediolateral physiological pattern of some sort, in relation to which different developmental potencies are realized.

A further study of eye development in chorio-allantoic grafts of late primitive-streak and head-process stages has shown that the potentiality for eye development is realized in the grafts from an area of field about the

primitive pit in late primitive-streak stages and later at the anterior end of the head process. Frequency of eye development is higher in grafts from the median than in those from the lateral regions of this field, and in those from the left side than in those from the right, as in Rawles's experiments.

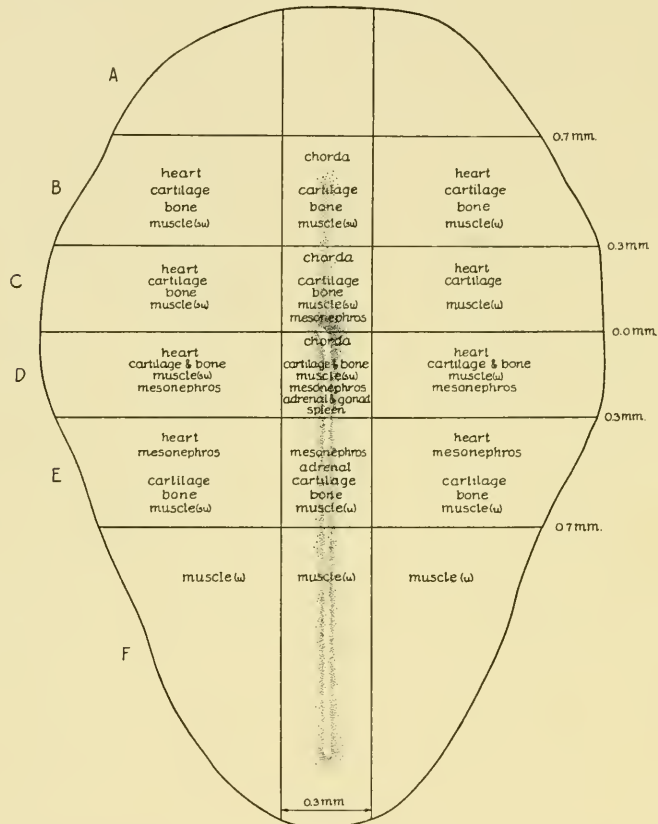


FIG. 168.—Regional distribution of development of mesodermal structures in chorio-allantoic grafts of head-process blastoderm of chick; measurements as in Fig. 167; *s*, striated muscle; *u*, nonstriated muscle (from Rawles, 1936; prepared by Willier and Rawles).

In later stages capacity for eye development becomes greater laterally than in the median region. The higher frequency in median than in lateral grafts of organs normally lateral, such as eye and ear, is like eye frequency in amphibian development at certain stages and, like that, suggests precedence of the median region in attainment of a certain physiological condi-

tion (pp. 282-85). The map of Figure 170 shows in a different way regions from which tissues of certain organs differentiate in chorio-allantoic grafts.

The results of grafting and explantation show a very considerable capacity for reconstitution in many of the isolated parts and a general but

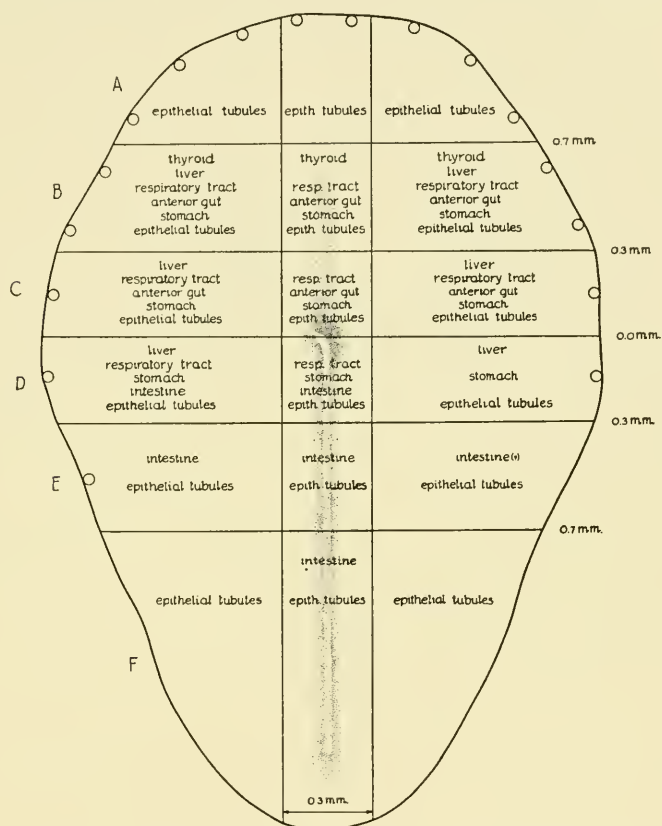


FIG. 169.—Regional distribution of development of entodermal structures in chorio-allantoic grafts of head-process blastoderm of chick; circles at margin indicate pieces from which "primordial germ cells" were obtained; measurements as in Fig. 167 (from Rawles, 1936, prepared by Willier and Rawles).

definite relation of character of reconstitution to a spatial pattern of some sort. It seems evident that neural tissue can develop without induction and that in earlier stages the primitive streak may reconstitute from regions lateral to those in which it normally originates; this is perhaps largely a matter of continuation of the cell movements concerned in formation

of the streak. It appears probable, however, that in many of the experiments full realization of developmental capacities may be limited or inhibited by the experimental conditions. The chorio-allantoic grafts of the smaller blastoderm pieces do not give rise to orderly definite embryos of

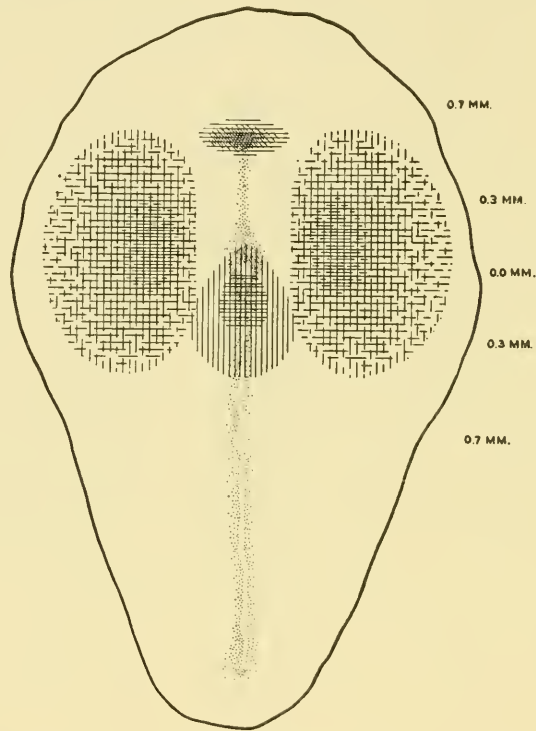


FIG. 170.—Map showing by shading certain organ areas of chick at head-process stage, as defined by chorio-allantoic grafts. In the median region at the anterior end of the head process is the eye area; heart develops from the large lateral areas; between them and median is the mesonephric area and central within it a cross-lined area representing the adrenal-gonad area; depth of shading in the various areas represents roughly intensity or degree of developmental potency for the organ concerned under the conditions of experiment (prepared by Willier and Rawles, kindness of Dr. Willier).

small size or even to axiate partial forms, but to tissue complexes; and many of them fail to develop at all, though they presumably possess the same potencies as others that do develop. Such failures may, of course, be due to incidental conditions, injury in isolation and grafting, inadequate attachment, inadequate blood supply, etc. But other factors are apparently concerned. Even when whole blastoderms of primitive-streak, head-

process, and early somite stages are grafted, parts posterior to the mesonephros do not develop in any case (Willier and Rawles, 1931). Also, grafted pieces from the more posterior levels of advanced primitive-streak stages show, at best, only slight differentiation. The absence of posterior parts in grafted whole blastoderms suggests that development is perhaps unable to proceed beyond a certain stage on the chorio-allantois. Size of piece may also play a part in determining occurrence and character of development of grafted pieces, as is maintained by Murray and Selby (1930). In postembryonic grafting in the lower invertebrates a larger piece very generally persists and develops more frequently than a smaller. The smaller fraction of gradient pattern in the smaller piece is less effective than a larger fraction; but even small pieces from a high gradient-level, grafted into a low level, may persist and develop. Similar questions arise with regard to explantation. The explanted whole blastoderm may develop quite normally up to a certain stage, at which development stops and death follows (Waddington, 1932). Interference with the cell migrations may also be an important factor in limiting realization of developmental capacities. Explanted transverse strips of definitive primitive streak and head-process blastoderms develop axial parts from the node-level and anterior regions, but development of levels of the streak is interfered with by the transverse sectioning (Rudnick, 1938*a*). The marked retardation of development in explants of pieces of early blastoderms and the disappearance of the streak structure from streak stages (Rudnick, 1938*b*) suggests that developmental potencies of these pieces are far from realized in these experiments. This may be due not to lack of organization but to the high susceptibility of these stages to the conditions of explantation. According to Butler (1935), the posterior quadrant of the unincubated blastoderm, from which the primitive streak later develops, gives rise to axial organs in chorio-allantoic graft; but in somewhat later stages the region of the early streak in explants develops only erythroblasts (Rudnick, 1938*b*). In spite of the many positive results of experiment and the advance in knowledge of avian development due to them, it still seems possible that developmental capacities of at least some parts of the blastoderm may be greater than experiment has shown. Some of the experimental data suggest that the gradient pattern of later streak and more advanced stages develops gradually in the blastoderm and that the apparent lack of organization in early stages may be lack or inadequacy of this pattern, but they throw no light on the problem of its origin.

EMBRYONIC DUPLICATIONS AND POLYEMBRYONY

Differential inhibition of embryonic stages not infrequently results in duplication or multiplication of parts or axes and sometimes in development of more than one individual from a single egg or early embryo. Examples are the duplications in insect development resulting from differential inhibition by cyanide (p. 518), polyembryony in fishes after inhibition by low temperature (Stockard, 1921) and by exposure to ultra-violet light (Hinrichs and Genther, 1931), and duplications in chick embryos resulting from various inhibiting conditions. Partial duplications in annelids and other forms may also result from inhibiting conditions. In these cases there is apparently a decrease in dominance with physiological isolation of regions normally subordinate. Temporary inhibition sufficient to prevent rapid recovery of the original dominance followed by return to natural conditions, permitting parts originally subordinate to become dominant, is apparently most effective in producing these duplications.

Duplications and multiplications, ranging from bifurcations, through all degrees of teratological duplications and multiplications and complete twins from single eggs, to development of many, even hundreds, perhaps thousands of embryos from a single egg, occur without experimental interference. Except in a few forms, complete and partial twinning appear only occasionally, as do the teratological forms, and many of them probably result from inhibiting conditions. The extreme types of polyembryony are usually normal characteristics of the species concerned. The natural polyembryonies raise interesting questions concerning developmental pattern, but at present it is possible in most cases only to call attention to some of them and to suggest possibilities.

Polyembryony has been observed in various coelenterates as a consequence of separation of blastomeres and blastomere groups, a "blastomere anarchy," as Metschnikoff describes it.¹⁹ These cases may be results of slightly inhibiting laboratory conditions or other unfavorable conditions. Low oxygen in standing water may be sufficiently inhibitory to obliterate any pattern originally present in early embryonic stages, with resulting isolation of cells or cell groups, and the differential between free surface and surface in contact may determine new polarities in the isolates, or they may reconstitute from the part of the original pattern persisting in them.

In several genera of bryozoa the blastomeres of earlier stages are apparently completely separated, and follicle cells may lie between them.

¹⁹ See, e.g., Busch, 1851; Haeckel, 1881; Metschnikoff, 1886.

Later they form a compact rounded mass of cells with more or less definite outer layer but without any indication of definite embryonic structure or polarity. Still later, outgrowths from this primary embryo develop, sometimes becoming elongated, finger-like extensions; from the tips of these outgrowths cell masses, varying somewhat in size, separate as secondary embryos. These may develop or in certain species give rise to further outgrowths from which tertiary embryos develop.²⁰ There is no indication of polarity in the early stages of these masses. According to Harmer, however, the outgrowths develop only toward the distal end of the ovicell in certain species of *Crisia*. This perhaps indicates presence of a polar pattern determined by some differential in the ovicell. In other species of *Crisia* Robertson finds outgrowths developing in any direction. Each outgrowth probably represents a localized region of increased cellular activity like a bud, the localizing factor being apparently more or less fortuitous, and each mass separating from the outgrowth may develop polar pattern from the part of the longitudinal pattern of the outgrowth persisting in it; but, even if this is the case, the origin of ventrodorsality remains obscure. That axiate physiological pattern is acquired at some time in the course of this polyembryonic development and in relation to some differential or differentials in conditions and that a definite egg pattern is not necessary for development of an axiate individual in these forms is highly probable.

Development of two whole embryos from a single egg and various degrees of embryonic twinning, involving anterior or posterior duplications or both, are not infrequent in oligochete annelids.²¹ These duplications, like some experimental duplications discussed in the following chapter (pp. 556-61), are of special interest as indicating that annelid development is not as completely a "mosaic" of self-differentiating parts, as often assumed.

In certain parasitic hymenoptera there are extreme degrees of polyembryony, although maturation and early cleavage suggest presence of axiate pattern of some sort and degree. If such pattern is present, there is no evidence that it persists or is effective in later stages. By continued cleavage the primary embryo becomes a solid mass of cells, apparently without definite arrangement or visible pattern. Constrictions divide this embryo into a number of cell masses, constituting the polygerm; and these primary masses divide to form secondary masses; these, in turn, to form

²⁰ Harmer, 1893, 1896, 1898; A. Robertson, 1903.

²¹ Kleinenberg, 1879; Vejnovsky, 1883-92; Weber, 1917; Welch, 1921; Penners, 1924a.

tertiary; etc.²² The cell masses may differ considerably in size, and, according to Patterson (1921), an embryo may develop from a single cell. On the other hand, many masses fail to develop in some species, and some give rise to asexual, nonviable individuals. There seems to be no common orientation of the axes of embryos in the polygerm, nor do all embryos develop at the same time or from cell masses of the same generation. There is no evidence of persistence of axiate pattern through all the divisions of the polygerm, and the apparently fortuitous character of the divisions suggests that, if pattern was originally present, it has been obliterated. Can axiate pattern originate autonomously in the final generations of cell masses? If a gradient pattern, a molecular pattern, or a spatial pattern of any kind determining axiate organization of the individual insect does arise *de novo*, it is difficult to conceive how this is possible, genetically or otherwise, except in relation to some initiating factor external to the mass concerned. Differentials in oxygen supply or in CO₂ accumulation and perhaps potential differences in the parent body and between the masses may be factors in initiating pattern. Some of the figures given in papers cited above suggest that the final polarities of embryos may be determined by a differential in their local environment—for example, between the wall and the interior of the polygerm—but the question is not discussed by the authors. Failure of some masses to develop may be due to absence or inadequacy of axiate pattern rather than to nutritive conditions, as suggested. Development of masses of any generation and failure of many to develop suggests that growth and division of the masses continues until a mass acquires a pattern adequate for development. *In vitro* cultivation of the polygerms or cell masses, if found to be possible, may give some information concerning their developmental physiology.

In most vertebrates polyembryony is only occasional under natural conditions and is limited to various degrees of twinning, ranging from all degrees of teratological duplication to conjoined twins, equal or unequal in development, and completely separate duplicate or identical twins. The descriptive literature of vertebrate teratology is voluminous, and the question whether duplications and multiple forms result from fusion of originally separate embryonic primordia or from division of a single primordium has been discussed for many years. However, continued study of the teratological forms and of complete twins and the experimental embryonic reconstitutions have established beyond question the origin

²² Marchal, 1904; Silvestri, 1905, 1906, 1908; Patterson, 1915, 1921; Gatenby, 1918; Leiby, 1922; and literature cited by these authors.

of many of these forms from a single egg or an originally single embryo.²³ Evidently such duplications are results of an agamic reproduction—a budding or fission with reconstitution of pattern—occurring in early embryonic stages; but conditions concerned in their origins can usually only be inferred or guessed at.

Experiments, both on embryonic and postembryonic stages of plants and animals, show that decrease of dominance by exposure to inhibiting conditions may result in a greater or less degree of physiological isolation of previously subordinate parts, in establishment of new dominant regions, and, in some forms, with proper experimental procedure, in complete obliteration of the original polarity and dominance. That somewhat similar factors are concerned in many cases of nonexperimental embryonic duplication, even in higher vertebrates, seems probable, as suggested by Newman (1917a, 1923). Toxic or other conditions inhibiting developmental activity may weaken dominance and polarity to such an extent that new polarities and dominant regions may arise in less susceptible regions or after the inhibiting conditions cease to act, either in relation to what remains of the original pattern or in reaction to local differentials. The high frequency of duplications with definite relation to the original symmetry pattern suggests that this often plays a part in determining localization of the new dominant regions. Partial axial duplication may, of course, also result from mechanical division of a dominant region—for example, split tails, limb buds, etc. Accidental or pathological conditions may determine some duplications in this way.

Even among the mammals, however, polyembryony is not limited to teratological forms and occasional identical twins. In two species of armadillo development is normally polyembryonic. With few exceptions four embryos develop from a single egg in the nine-banded armadillo, *Dasypus* (= *Tatusia*) *novemcinctus*,²⁴ and from six to twelve, usually eight or nine, in *D. hybridus* (Fernandez, 1909). The quadruplets of *D. novemcinctus* arise by two successive agamic reconstitutions of two embryonic primordia from a single one. According to Patterson, these are primary and secondary buddings, but Newman regards them as fissions. Since they involve the origin of new axiate patterns, they seem to resemble buds more closely than fissions. The new patterns apparently originate in definite relation

²³ See, e.g., Klaussner, 1890; Dareste, 1891; Bateson, 1894; H. H. Wilder, 1904, 1908; Schwalbe, 1907; Gemmill, 1912; Newman, 1917a, 1923; and citations by these authors.

²⁴ Newman and Patterson, 1909, 1910, 1911; Patterson, 1912, 1913; Newman, 1917a, 1923.

to environmental factors, for the first duplication is right and left with respect to the parent, on the two sides of the primary embryonic vesicle toward the openings of the Fallopian tubes, and the second duplication is in definite relation to the first.

Newman (1923) holds that this polyembryony is associated with occurrence of a quiescent period in development, during which the original developmental pattern is, to a large extent, obliterated; and with renewal of developmental activity the reaction to, and the determination of, a new pattern by environmental differentials within the uterus results. Even after this, however, the integrating factor is not adequate to prevent a second physiological isolation of parts; consequently, four embryos are formed. The entire sequence of reconstitutions in *D. hybridus* has not been followed, but the irregular arrangement and variable number of embryos in that species indicate that some of the embryonic primordia bud or divide more than others. There seems to be no doubt that this type of polyembryony involves repeated obliteration and determination of developmental patterns, and its occurrence in the mammals suggests that under proper conditions pattern in various other eggs may not be as stably determined as commonly assumed.

FUSIONS OF EMBRYONIC AND LARVAL INDIVIDUALS

Larvae of the sponge *Lissodendoryx*, merely brought into contact at certain stages, fuse readily into masses consisting of indefinite numbers of larvae in which all evidences of individual form and polarity disappear. These masses, in contact with a solid substratum, may metamorphose into perfect sponges (H. V. Wilson, 1907). Evidently there is complete obliteration of the original larval patterns in these masses, and the polarity of the resulting sponge is determined anew by an environmental differential, probably the differential arising between free surface and surface in contact with the substrate, since development of the osculum or oscula is on the free surface, as in aggregates of dissociated sponge cells (p. 418). Like the cell aggregates, these fusion masses would be interesting material for other experiments on determination of pattern.

Normal planulae from fused blastulae of the medusa *Mitrocoma* have been described, but nothing is known concerning possible changes in pattern. Fused cell masses resulting from cleavage in calcium-free sea water of eggs of the nemertean *Cerebratulus* may, after return to normal sea water, develop into giant pilidium larvae with multiple organs (Yatsu, 1910c). Apparently in these the original pattern is not completely oblit-

erated; but whether, or to what extent, it may be altered, is not known. Fusion among the several eggs in a capsule, either before or during cleavage, has been observed in another nemertean, *Lincois ruber*; and fusion of two eggs may result in normal gastrulae with cells approximately double size (Nusbaum und Ochsner, 1913).

The giant eggs of *Ascaris megalcephala* undoubtedly result from fusion of two or more eggs. Completeness of fusion varies widely; the giant eggs may be doubly fertilized, form two sets of polar bodies at different points, and show more or less double cleavage; or in some cases they may develop as a single individual.²⁵ Apparently the variations depend on the relations of the apicobasal axes, on the degree of physical union, and perhaps on other factors. Supposedly, development as a single individual occurs only when polarities of fusing eggs are parallel and identical in direction.

Fused unfertilized and fertilized sea-urchin eggs and early developmental stages give forms ranging through various degrees of twinning to single individuals. Skeletal duplications or excessive skeletal development and duplications of the archenteron are frequent.²⁶ Fusions of more than two individuals are usually highly abnormal and die early. Single giant larvae may develop from fusions with axes known not to be parallel, and twinning may occur when axes are parallel. Extensive shiftings and rotations of the components, either toward or away from parallelism, may take place. The larger component may dominate the smaller to such a degree that suppression of its development, reduction in size with translocation of cells, and even complete incorporation into the body of the dominant member may result. Translocation of mesenchyme cells to the dominant member indicates that the regions of its ectoderm which determine localization of mesenchyme are more effective than those of the subordinate member. At present it appears difficult to interpret the observed results except in terms of dynamic factors. Investigation by means of differential dye reduction of the gradient relations and the changes which they undergo in these fusions, particularly in cases of suppression and absorption of one member, would undoubtedly be of interest.

First cleavage stages of *Ascidiella aspersa* united in various orientations develop as more or less completely double forms, often with organ anomalies and dislocations, or as forms apparently single externally but more

²⁵ Sala, 1895; Zur Strassen, 1898, 1906; Kautsch, 1913.

²⁶ Driesch, 1893, 1900, 1903, 1910; Morgan, 1895c; Garbowski, 1904; Bierens de Haan, 1913a, b; Goldfarb, 1914b, 1915, 1917; von Ubisch, 1925a; Schleip, 1929, pp. 484-95.

or less completely duplicated internally; or in a single case among more than a hundred developing, a single individual resulted from union with apical pole of one component in contact with basal pole of the other (von Ubisch, 1938*b*). The author holds that the general organ-forming regions develop according to their prospective significance but that within them there may be extensive reconstitution. Two such regions may give rise to a single organ system, or one may form two systems.

Homoplastic and heteroplastic fusions of two two-cell stages have been accomplished with interesting results in species of the urodele *Triton*. After removal of membranes the blastomeres of the two-cell stage become almost spherical and are connected only by small areas. At this stage one pair of naked cells is laid crosswise on the other, and the four cells come gradually to lie in a plane, the blastomeres of one component alternating with those of the other. From these fusions one to four axial systems may develop.²⁷ A single embryo resulting from a heteroplastic fusion is a chimera, that is, composed of cells of two species. The results of these experiments are interpreted in terms of the relation of the first cleavage plane to the median plane and the consequent positions in the fused pair of the dorsal inductor tissue, assuming that this inductor region is already more or less definitely localized and determined at the two-cell stage. Since the first cleavage is known to make any angle with the median plane, the dorsal inductor region of each two-cell stage may be divided by it into equal or unequal parts or be entirely in one cell, and the relation of cleavage plane and median plane may be different in the two fused components. Consequently, neural induction and embryonic axes will appear in the fused pair in various positions and combinations of parts. The results agree with expectation and are in accord with other experiments (chap. xii) in indicating that a part of the inductor may reconstitute to a whole, that induction is not species-specific, and that such symmetry pattern as may be present at the two-cell stage may undergo extensive alteration in the reconstitutions resulting from the fusions.

CONCLUSION

It appears that embryonic reconstitutions in many animals do not differ very greatly from reconstitutions in adults of the lower invertebrates, except that they are usually more narrowly limited. They commonly show definite relations to the original pattern, but sometimes that is completely obliterated and new pattern determined. The embryonic

²⁷ Mangold, 1920; Mangold und Seidel, 1927; also Spemann, 1938, pp. 271-77.

reconstitutions provide further evidence in support of the view that the effective factors in the developmental patterns of early stages must be sought in the dynamics of living protoplasts rather than in concentrations of hypothetical formative substances. As we pass from later to earlier stages of embryonic development, there is progressively less evidence of regional specificity, and in some eggs at the beginning of embryonic development there is apparently little or nothing more than quantitative gradient pattern. In others regional specificities are already present at the time of the first cleavage, though evidences of gradient pattern may still be present. Some of these, in which the original pattern tends to persist with little or no change in isolated parts and development appears to be more or less completely a mosaic of independent, self-differentiating systems, are discussed in the following chapter.

CHAPTER XIV

CLEAVAGE AND DEVELOPMENTAL PATTERN

IF NUCLEI are primarily alike as regards hereditary potentialities, the physiological basis of embryonic developmental patterns must be sought in the egg cytoplasm; and the question whether, or to what extent, the widely different cleavage patterns of different animals are related to developmental pattern arises. Some cleavage patterns—for example, those of annelids, mollusks (except cephalopods), ascidians, and some other forms—appear to be intimately related to developmental pattern; they are highly determinate, that is, certain cells give rise to certain organs or parts by a definite cell lineage, and at least some of the cells, when isolated, are capable of continuing development for a time with little or no change. For this reason earlier stages of these forms have often been regarded as “mosaics” of independently developing parts. At the other extreme are completely indeterminate cleavage patterns without any definite relation to developmental pattern, as in polyembryonic bryozoa, in insects and probably most other arthropods, and in meroblastic vertebrate eggs. Between these extremes are various degrees of determinate character. In other, less specialized forms of development, budding, fission, and reconstitution of multicellular forms the single cell has no definite relation to developmental pattern. This suggests that the more highly determinate types of cleavage may be expressions of a certain degree of determination or differentiation of regions of the undivided egg. This chapter is largely concerned with the more highly determinate cleavage patterns and with questions regarding their mosaic character and relation to pattern of the organism.

SPIRAL CLEAVAGE PATTERN

As far as mitotic spindles and cleavage planes are concerned, so-called “spiral cleavage” is actually oblique. To an observer in the egg axis with head toward the apical pole the spindles are inclined with upper poles to right (dextrotropic) or to left (leiotropic), and the upper cell is obliquely dextral or sinistral to the lower. This type of cleavage has been called

“spiral” because it may be conceived as resulting from a spiral twisting of planes of radial or bilateral symmetry into surfaces forming a spiral about the polar egg axis. There is, however, no evidence of such twisting, but the term “spiral cleavage” has been so generally employed that it is used here. In general, this pattern of cleavage gives way sooner or later to more or less bilateral patterns; but as long as it persists, each spindle is approximately perpendicular to that of the preceding division of the cell concerned, and consequently successive cleavages are alternately dextro-tropic and leiotropic. Because this form of cleavage occurs in the earlier stages of polyclad turbellaria, annelids, gephyreans, and mollusks, except cephalopods, and because it is sufficiently determinate in character to permit the following of cell lineage from first cleavage to certain regions or organs of the larva or later embryos, it has received much attention and has been largely responsible for the concept of ontogeny as a mosaic and for theories of the phylogenetic significance of cleavage and of cell homologies.¹

Various forms and stages of spiral cleavage are shown in Figures 171-76. Different terminologies for designating the blastomeres have been used, but the following has become more or less standard. The first cleavage is meridional and may be equal (Fig. 171, *A*) or unequal (Figs. 172, *A*; 175, *B*). When it is unequal, the smaller cell is *AB*, the larger, *CD*. Second cleavage is also meridional and divides *AB* equally into *A* and *B*; and in those forms in which the first cleavage is distinctly unequal, the second divides *CD* into a smaller cell, *C*, and a larger, *D*. In polyclads and nemerteans the four quadrants are all alike and indistinguishable (Fig. 171, *B*). In the third cleavage the first quartet of “micromeres” (*1a-1d*) separates from four basal “macromeres” (*1A-1D*). The so-called “micromeres” are usually smaller than the basal macromeres (Figs. 172, *C*; 173, *A*; 174, *A*, *C*) but in some forms are equal in size to, or larger than, the latter (Fig. 171, *C*); and definite size differences characteristic for the species are often present, *1d* and often *1c* being larger than *1a* and *1b* (Figs. 172,

¹ For more or less extensive descriptive studies of spiral cleavage see the following: *Polyclads*: Hallez, 1879; A. Lang, 1884; E. B. Wilson, 1898; Surface, 1907. *Nemerteans*: Zeleny, 1904. *Annelids*: Whitman, 1878; Salensky, 1882-83, 1885; Vejdovsky, 1883, 1892; Hatschek, 1886; E. B. Wilson, 1892; von Wistinghausen, 1893; Wheeler, 1897; Mead, 1897; Eisig, 1898; Child, 1900; Treadwell, 1901; Schleip, 1914a; Penners, 1922, 1923, 1924a, *b*, 1925. *Gephyreans*: Griffin, 1899; J. C. Torrey, 1903; Gerould, 1906. *Mollusks*: Rabl, 1879; Hatschek, 1881; Blochmann, 1882, 1883; Heymons, 1893; Kofoid, 1895; F. R. Lillie, 1895; Meisenheimer, 1896, 1901; Conklin, 1897, 1907; Drew, 1899; Heath, 1899; S. J. Holmes, 1900; Robert, 1903; E. B. Wilson, 1904; Wiersejski, 1906.

C; 173, A; 174, A). Division of both apical and basal quartets follows, the apical usually preceding and giving rise to $1a^1$, $1a^2-1d^1$, and $1d^2$, the basal to $2a-2d$ and $2A-2D$. In most forms four or five quartets of micromeres are formed, each farther from the apical pole than the preceding, before the spiral pattern is altered or obliterated in the cells concerned. Since the spindles are alternately dextrotropic and leiotropic, the blastomeres interlock; and when the cleavages are equal or nearly equal, the planes of contact are essentially similar to those resulting from surface tension, as in a mass of soap bubbles (Robert, 1903). As far as known,

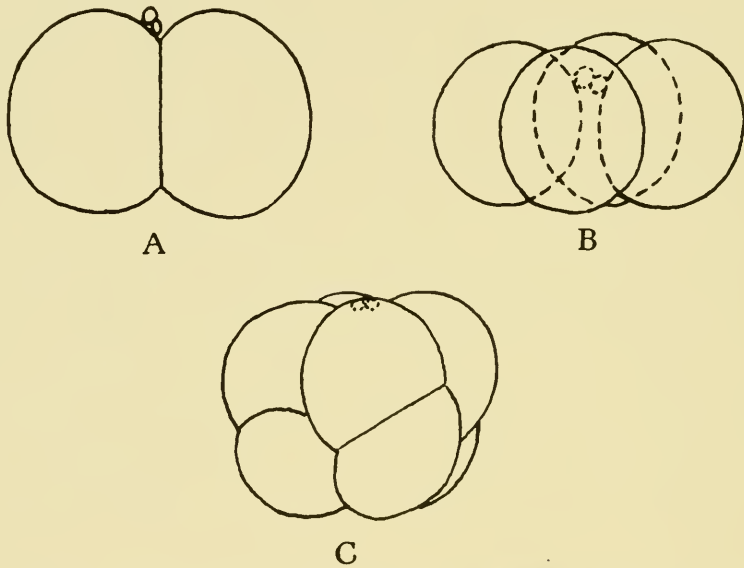


FIG. 171, A-C.—Early cleavages of the nemertean *Cerebratulus* (after Zeleny, 1904)

certain descendants of the first quartet form the whole or part of the prototroch; but numbers and origins of cells in prototrochs of different species differ widely, and the series of divisions leading to its formation also differ, or, when similar, are similar because they are spiral cleavages. And in forms without prototroch, cells equivalent in origin to the trochoblasts form other parts of the ectoderm. Later divisions of the pretrochal cells about the apical pole also differ in different species. The second quartet of micromeres was regarded by Lang (1884) as entirely mesodermal in polyclads; but, according to E. B. Wilson (1898) and Surface (1907), these cells in all four quadrants give rise to both ectoderm and mesoderm. In the gasteropod *Crepidula* three of these cells ($2a$, $2b$, $2c$)

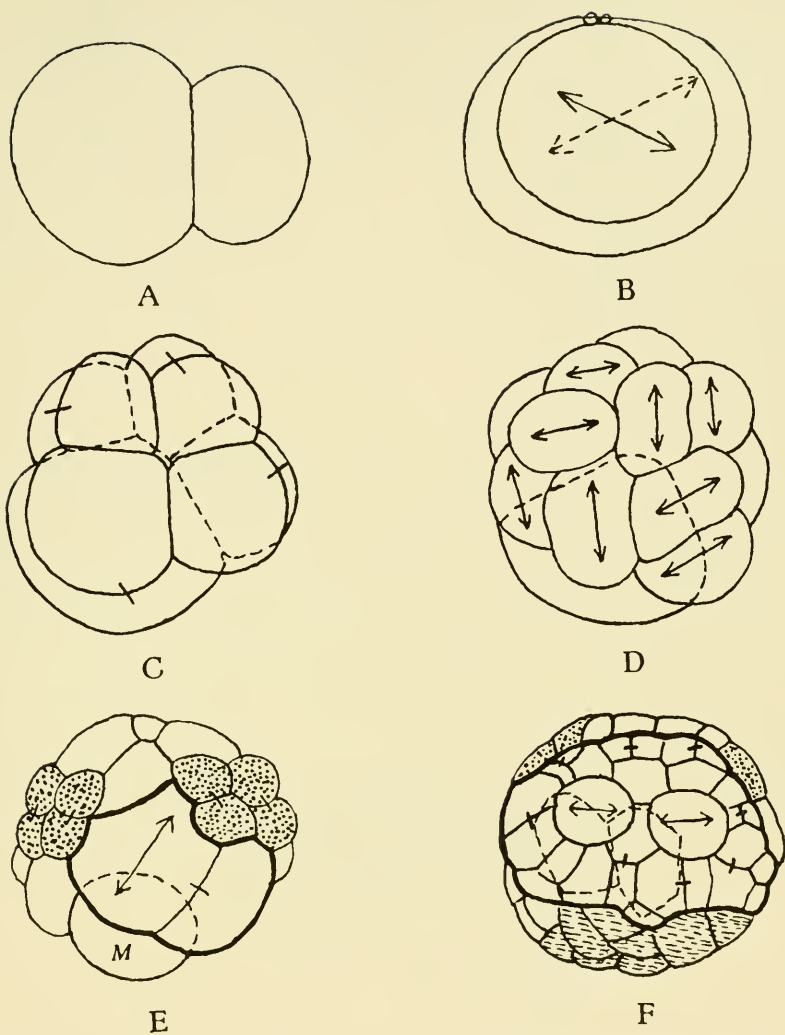


FIG. 172, A-F.—Cleavage stages of *Arenicola*. A, two-cell stage; B, second cleavage; C, eight-cell stage; D, second division of first quartet of micromeres and of the cell *2d* (first somatoblast); E, primary trochoblasts, shaded, and two-cell stage of *2d*, surrounded by heavy line, also the cell *M* (*4d*), the second somatoblast or mesoblast; F, later stage, showing development of somatic plate, surrounded by heavy line, the two mesoblasts, outlined in broken line, already in blastocoel, and entoderm cells indicated by parallel broken lines (after Child, 1900).

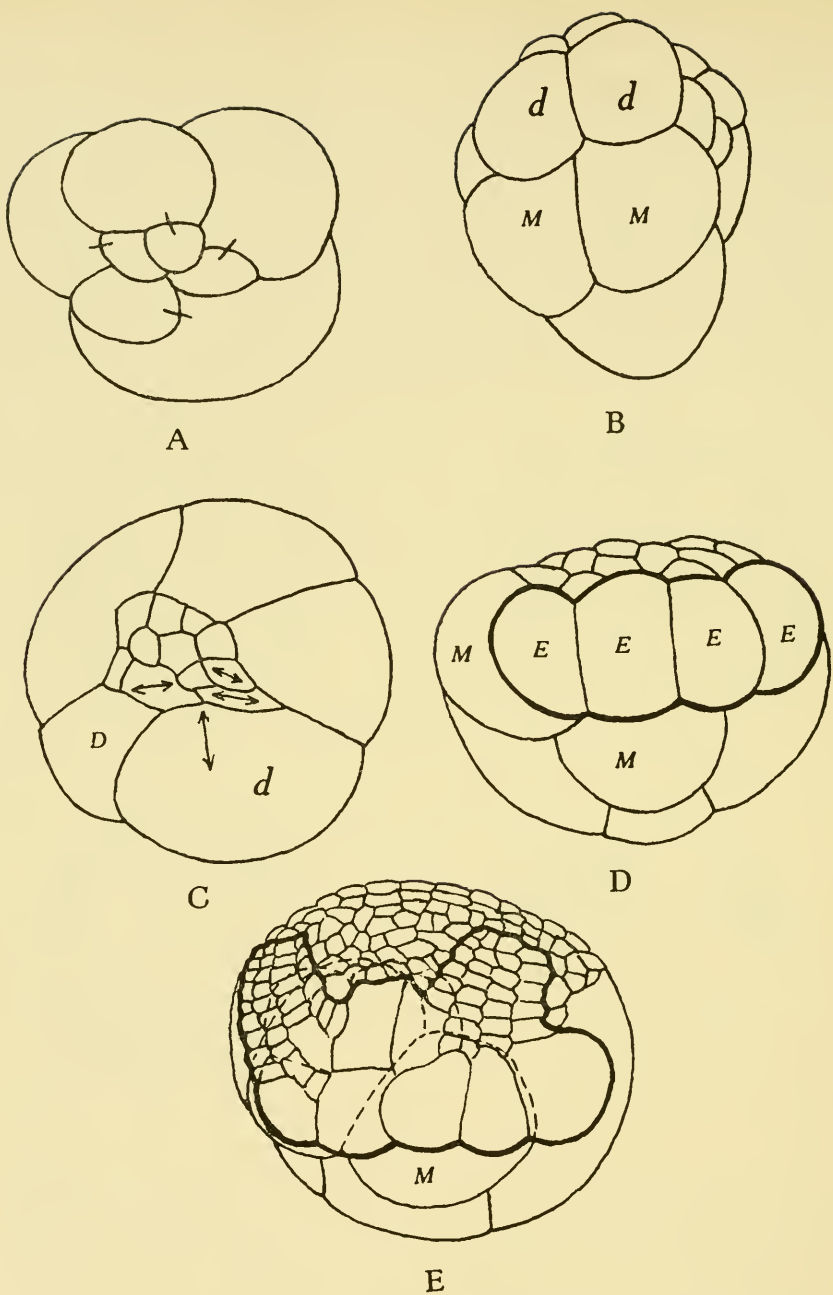


FIG. 173, *A*, *B*.—Cleavage stages of *Tubifex*. *A*, eight-cell stage. *B*, later stage, showing $2d$ and $4d$ (M) (after Penners, 1922). *C*–*E*, cleavage stages of leech, *Clepsine* (= *Glossiphonia*); *C*, early stage, showing $2d$ (first somatoblast) and $3D$ (second somatoblast or mesoblast); *D* later stage, showing E , E , E , E , products of $2d$, and M , M , the mesoblasts, products of $3D$; E , ectodermal teloblasts and germ bands from $2d$, surrounded by heavy line, and mesoblasts, M , M (after Schleip, 1914a).

give rise to ectoderm and mesoderm, and the fourth (*2d*) is entirely ectodermal (Conklin, 1897). In *Unio* three are wholly ectodermal, only one

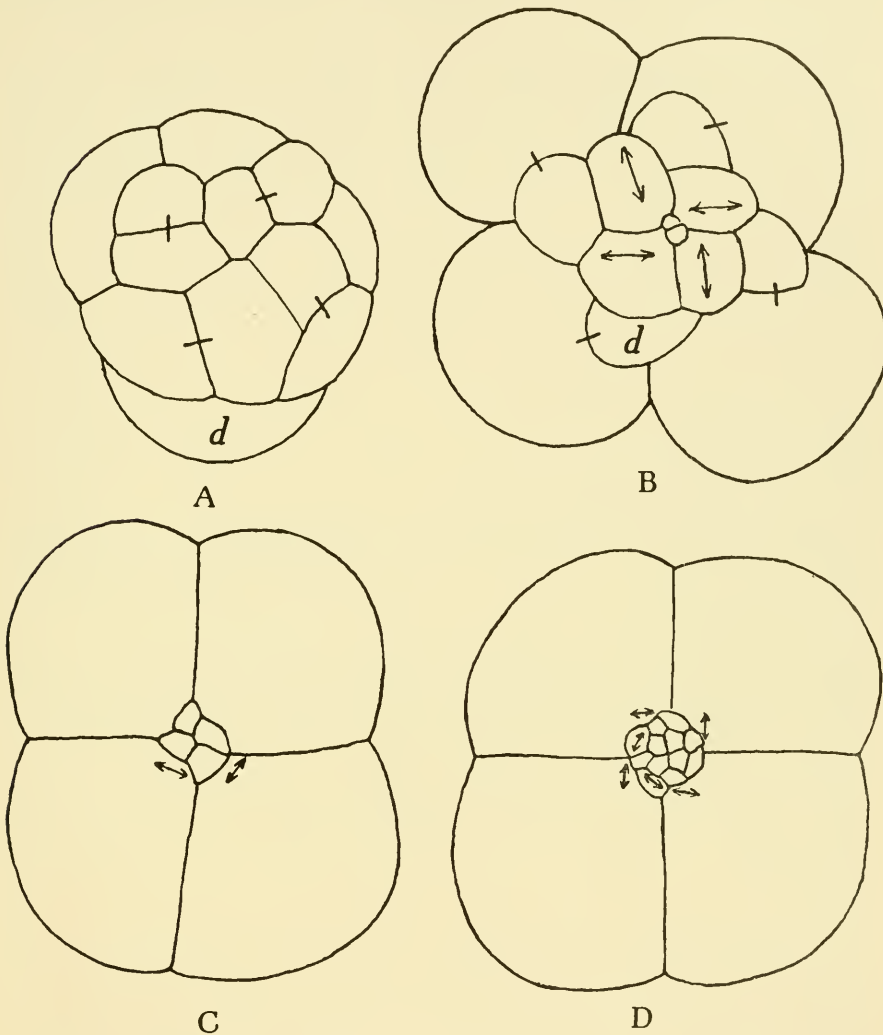


FIG. 174. A-D.—Cleavage stages of mollusks. A, *Unio*, first and second quartets, *2d* largest (after F. R. Lillie, 1895); B, *Crepidula*, *2d* no larger than other second quartet cells (after Conklin, 1897); C, D, *Fulgur*, first and second quartets, including *2d*, all relatively very small (after Conklin, 1907).

(*2b*) ectomesodermal (F. R. Lillie, 1895); while in polychetes all are apparently wholly ectodermal, except for the doubtful origin of mesoderm from cells of the second or third quartet of *Aricia* (Wilson, 1898). If the

origin of mesoderm from cells of the second or of the third quartet is an ancestral reminiscence, as has been suggested, it might be expected to appear in greater degree in annelids than in mollusks, but apparently it does not.

The cell *2d* is similar in size to other members of the second quartet, and its descendants apparently give rise to no more ectoderm than the other cells of the quartet in polyclads and nemerteans; but in annelids it is usually larger than other members of the quartet, is called "first somatoblast," and its descendants constitute most of the trunk ectoderm, giving rise in oligochetes and leeches to the ectodermal teloblasts from which the ectodermal germ bands develop (Figs. 172, *D, E, F*; 173, *B-E*), but the cleavage patterns by which these results are accomplished differ in almost every species studied. Among the mollusks the cell *2d* may be much larger than other cells of the second quartet, as in *Unio* (Fig. 174, *A*), or the same size as others (Fig. 174, *B, D*). In *Unio* shell gland and pedal ectoderm are derived from it (Lillie, 1895); and that this is also true for *Crepidula* is held by Conklin (1897, 1907), in spite of the great difference in size of the cell and time of appearance of shell gland as compared with *Unio*. The third quartet (*3a-3d*) is regarded as ectodermal, except that *3b* is said to give rise to entoderm in the oligochete *Tubifex* (Penners, 1922).

The cells *4a, 4b*, and *4c* of the fourth quartet become entoderm in cases followed to this stage. In annelids and mollusks *4d*, the second somatoblast, is much larger than other cells of the quartet and becomes mesoderm (*M* of Fig. 172, *E, F*, and of Fig. 173, *B, D, E*). In the leech *Clepsine*, according to Schleip (1914), *4D* also becomes mesoderm (Fig. 173, *D, E*); but in other forms it gives rise to entoderm, and what remains of the "macromeres" after formation of the fourth quartet also becomes entoderm.

In many annelid and mollusk eggs certain cytoplasmic regions are visibly distinguishable, either by absence of yolk or by presence of certain granules. These regions may apparently undergo definite changes in position in connection with maturation and fertilization and be distributed to particular cells during early cleavages. Very generally the amount of yolk and other granular inclusions increases basipetally from the apical polar region; but in many eggs a well-defined zone, aggregation, or ring of cytoplasm containing little or no yolk is present about the apical pole, either before maturation or appearing later; a second aggregation often appears about the basal pole. These polar plasms are, or become, very definitely localized in eggs of *Tubifex* (Penners, 1922) and *Clepsine* (Whit-

man, 1878; Schleip, 1914), which contain much yolk; in *Dentalium* eggs they appear less definitely bounded and are separated superficially by a pigmented, yolk-containing zone (E. B. Wilson, 1904). Some other eggs show one or both of them in differing degrees. In consequence of the definite character of cleavage these polar plasms are included in certain cells; in *Clepsine* both are entirely or almost entirely included in the cell *CD* by the first cleavage and in *D* by the second. Before the third cleavage the basal polar plasm migrates apically and unites with the apical plasm, and the entire mass remains in *1D* at the third cleavage and at the next division is divided between *2d* and *2D*. The ectodermal germ bands develop from descendants of *2d*, and the mesoderm bands from *4d* and *4D*. These derivatives of *D* constitute almost the whole mass of the cell, *3d* being small. Very similar behavior of the polar plasms has been described for oligochetes, and eggs in which polar plasms do not appear fail to develop or die in early stages and fail to develop somatoblasts (Vejdovsky, 1888; Penners, 1924*b*). Penners apparently believes that failure to develop and death result from absence of the polar plasms, but it seems possible that absence of polar plasms may result from physiological or pathological conditions and is only incidentally associated with developmental failure and death.

A temporary cytoplasmic yolk lobe or polar lobe appears basally in many mollusks and annelids at the first cleavage and in some species again at second and third cleavages. The three successive lobes of the first three cleavages of *Dentalium* are shown in Figure 175, *A-D*. Normally the lobe may become almost completely separated from the rest of the egg by constriction; but it is actually a part of one blastomere—*CD* in the first cleavage, *D* in the second, and *1D* in the third. In *Dentalium* it consists largely of the unpigmented basal plasm, but in some other forms it contains mostly yolk. However, in lobe-forming centrifuged eggs the lobe appears quite independently of the cytoplasmic stratification and may consequently differ in content in different individuals, according to the direction of stratification (p. 585). Its formation in connection with cell division and the flow of cytoplasmic substance into it as it enlarges led Boveri (1910*a*) to suggest that it represents a part of the cell not included in the sphere of influence of the adjoining aster; but the periodic form changes in isolated lobes of *Ilyanassa*, corresponding more or less closely to periods of the division cycle, occur in cytoplasm entirely isolated from the dividing cell (Morgan, 1933, 1935, 1936). Hydrostatic pressure of 220 atmospheres, applied in early stages of lobe formation, brings about withdrawal of the lobe and inhibition of cytoplasmic division in *Chae-*

topterus. This result, like Morgan's centrifuge experiments, indicates that the lobe is a cortical effect, associated with cytoplasmic division but largely independent of the mitotic apparatus (Pease, 1940). Experimentally the first lobe can be made to fuse with either of the cells of the two-cell stage in the mollusks *Dentalium* (Schleip, 1939, p. 208) and *Ilyanassa* (Morgan, 1936) and in the annelid *Sabellaria* (Novikoff, 1940, see footnote 8, p. 559), and the cell receiving it develops like a CD cell.

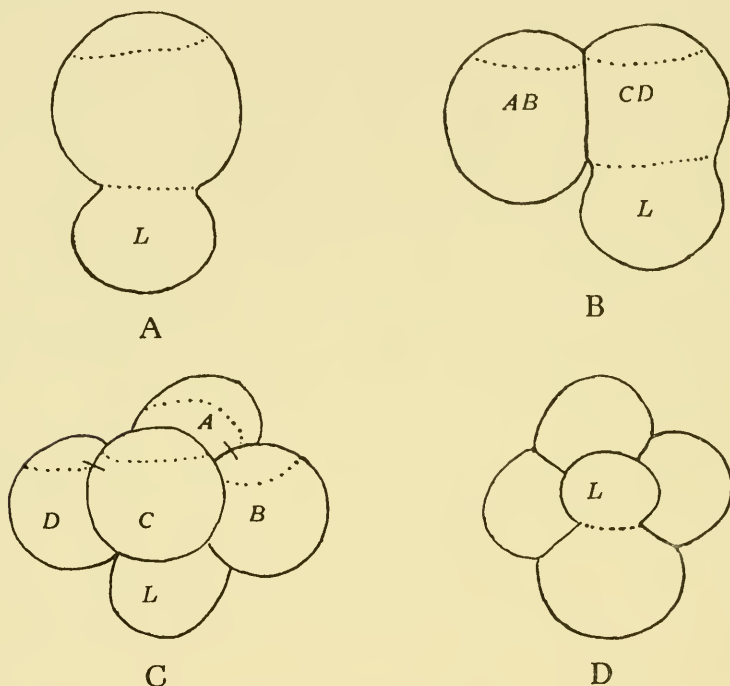


FIG. 175, A-D.—Early cleavages of *Dentalium*, to show the three successive polar lobes, L_1 - L_3 , and approximate boundaries of pigmented zone, indicated by dotted lines (after E. B. Wilson, 1904).

The earlier, purely descriptive studies of cell lineage in forms with spiral cleavage led to a further development of the hypothesis of "organ-forming germ regions" advanced by His (1874). The definite cytoplasmic localization, "precocious segregation," of different organ-forming substances and their accurate distribution by cleavages was postulated, and the concept of homology was applied to individual cells in the cleavage pattern, certain small cells in some forms being regarded as vestigial or rudimentary. According to these views, development in forms with spiral cleavage is a

mosaic, that is, it proceeds autonomously in the various self-differentiating parts.²

However, as data on different species accumulated, it became increasingly evident that supposed cell homologies were in many cases less exact than had been supposed. The first cleavage is said to be almost transverse to the median plane in some annelids (*Nereis*, *Chaetopterus*) and in some mollusks (*Crepidula*, *Umbrella*), while in certain polyclads (*Thysanozoon*, *Leptoplana*), other annelids (*Clepsine*, *Amphitrite*, *Arenicola*), and mollusks (*Planorbis*, *Unio*, *Trochus*) it is approximately 45° from the median plane. These differences necessitate the assumption of at least some differences in cellular localizations of different formative substances. The first cleavage may be equal or unequal, apparently without definite relation to the fates of the cells. There is apparently a relation between spiral cleavage and the later asymmetry in gasteropods: spirals of corresponding cleavages are reversed in direction in certain sinistral species.³ Conklin (1903*a*, *b*) suggested that this might result from reversal of polarity in eggs of these forms, but no evidence of reversal has been found. Moreover, spiral cleavage is not accompanied by any general asymmetry in polyclads, annelids, and pelecypods.

The later cleavages of the first quartet and the fates of some of the cells differ in different species. Certain descendants of the first quartet become cells of the prototroch in some species; while in others corresponding cells form, but there is no prototroch. The first somatoblast, *2d*, may be of the same size as other cells of the second quartet or much larger, and this difference in size is apparently without definite relation to the final relative size of the parts developing from it or to the stage at which they differentiate. Also, the later divisions of *2d*, as far as they have been followed, differ as regards directions and sizes of products in almost every species studied. Such differences are well shown in earlier stages of the somatic plate in *Arenicola*, the part surrounded by heavy line in Figure 172, *E* and *F*, and the ectodermal teloblasts and early germ bands of another annelid, *Clepsine*, similarly indicated in Figure 173, *D* and *E*. Some cells of the second quartet are said to give rise to both ectoderm and mesoderm in certain species; but in others, apparently only to ectoderm.

Perhaps the cell *4d*, the second somatoblast, which becomes mesoderm

² See, e.g., E. B. Wilson, 1892, 1894, 1896, 1898; F. R. Lillie, 1895; Mead, 1897; Conklin, 1897, 1898, 1907; Heath, 1899.

³ Crampton, 1894; S. J. Holmes, 1899, 1900; Wiersejski, 1906.

in annelids and mollusks, presents the best case for cell homology in these groups; but even here there are difficulties. According to Schleip, it gives rise to only one of the mesodermal bands in *Clepsine*, the other being formed from $4D$, which is entodermal in other forms. Moreover, $4d$ is apparently entodermal in polyclads. Actually there are very few, if any, exact cell homologies in the groups with spiral cleavage. In order to apply the concept of homology to the cells, we must, in any case, regard certain cleavages as accomplishing segregation and others as nonsegregating. Visibly different regions are often more or less exactly segregated in different cells, but the centrifuge experiments show that this segregation has little or no significance for development in most cases. If the principle of homology is applicable to egg cytoplasm, the homologies seem to be, at best, regional; and their more or less exact coincidence with cell boundaries appears to be an incidental or secondary result of regional cytoplasmic differences of some sort rather than fundamentally significant for development.

A particularly interesting feature of development with spiral cleavage is the gradual appearance of bilateral pattern in the cleavage pattern and its inexact character in early stages of most forms. Different species and different regions of the embryos provide various examples. The annelid first and second somatoblasts, $2d$ and $4d$, and in *Clepsine* also $4D$, are cases in point. The cell plate (somatic plate) resulting from divisions of $2d$ in polychetes extends laterally on each side from the dorsal region and gradually acquires a roughly bilateral form (Fig. 172, *F*), but up to an advanced stage most of the cleavages in it have not been bilateral. Moreover, the median plane of the two mesoblasts does not coincide with that of the ectoderm of the somatic plate (Fig. 172, *F*), but in later stages coincidence is gradually attained. In *Clepsine* bilateral pattern appears earlier in descendants of $2d$ (Fig. 173, *D*); but even after the germ bands begin to form, the arrangement of the teloblasts is not necessarily completely bilateral, and the median planes indicated by the ectodermal teloblasts and by the two mesoblasts are farther from coincidence than in polychetes (Fig. 173, *E*).

The pattern resulting from spiral cleavage is essentially quadriradial in polyclads, in nemerteans, as far as known, and in some annelids and mollusks. In this pattern there gradually appears a dorsiventral pattern with bilateral symmetry or, in the gasteropods, asymmetry. These facts, quite apart from experimental data, raise the question whether this form of development is as completely a mosaic as has been believed. Is it pos-

sible to account for the gradual replacement of the spiral and radial pattern by a bilateral or asymmetric pattern except in terms of an ordering and integrating factor, a wholeness of some sort which determines the gradual appearance of the new pattern? It seems beyond the bounds of probability that a pre-established harmony in a mosaic of independent cells or cell groups resulting from spiral cleavage could be so complete as to accomplish this result. Whatever the conditions determining spiral cleavage, they are apparently different from those determining ventro-dorsal or bilateral pattern. Even in the gasteropods, in which reversal of cleavage is associated with reversal of asymmetry, the cleavage pattern and the pattern of asymmetry are very different. Neither cell homologies, nor regional homologies, nor precocious segregation throw any light on the physiological factors concerned in spiral cleavage.

A physiological gradient pattern, at least in the polar axis, is indicated in some forms by differential susceptibility, differential dye reduction, and differences in hydrogen-ion concentration, and the differential in rate of cleavage also suggests a polar gradient (pp. 119, 545); but in many forms apicobasal pattern is apparently not merely a quantitative gradient but a pattern of more or less definitely localized material differences, sometimes directly visible in the living egg. This pattern, however, is apparently in large part or wholly an incidental and secondary result of the essential pattern and can be altered to an extreme degree by centrifuging without affecting development in most forms with spiral cleavage.

EMBRYONIC RECONSTITUTIONS IN FORMS WITH SPIRAL CLEAVAGE

NEMERTEANS

The nemerteans *Cerebratulus lacteus* and *C. marginatus* have a typical spiral cleavage; but development of isolated parts of eggs before and after fertilization shows, first, that they are not primarily mosaics and, second, that there is a progressive regional determination or stabilization with early cleavages.⁴ Nucleated or nonnucleated pieces of unfertilized eggs sectioned in various planes, can be fertilized, cleave like whole eggs and develop into normal dwarf pilidium larvae. According to Wilson, however, pieces less than about one-fourth of total egg volume do not give rise to complete larvae. Here, as in other embryonic and postembryonic reconstitutions, scale of organization is decreased in the isolated pieces; and in pieces below a certain size the scale may be larger than the piece, and partial forms may result.

⁴ E. B. Wilson, 1903c; Zeleny, 1904; Yatsu, 1910b, c.

Removal of parts of the egg between fertilization and first cleavage shows increasing disturbance of cleavage pattern the later the removal, but essentially normal larvae may develop. In isolated $1/2$ and $1/4$ blastomeres the cleavage pattern is a half or fourth of the whole, except as shifts in cell position occur; but normal larvae may still result (Wilson). Developmental effects of removal of parts of one or both blastomeres during or after first cleavage are slight (Yatsu). The apical quartet of the eight-cell stage gives rise to larvae with apical flagellum but without enteron; the basal quartet, to larvae with enteron but no apical organ (Zeleny). Apical and basal parts of blastulae isolated by section show much the same differences (Wilson). The three experimenters conclude that there is progressive apicobasal localization of formative materials during early cleavages. More recent experiments agree in general with these results. Isolated blastomeres of the two-cell stage may form complete, symmetrical larvae, an extensive reconstitution. The four apical or the four basal cells of the eight-cell stage, when isolated, give rise to apical and basal partial forms; and isolated rings of cells an_1 , an_2 , veg_1 , and veg_2 , of the sixteen-cell stage,⁵ and various combinations of these rings, develop as if the other blastomeres were present (Hörstadius, 1937*b*). Nothing is known at present concerning gradients in the nemertean egg; but the unfertilized egg evidently possess a polarity, and its high reconstitutive capacity suggests that this polarity is a predominantly quantitative differential at that stage but undergoes progressive regional differentiation in early development. Certainly neither the egg nor early cleavage stages are mosaics.

DUPLICATIONS AND OTHER RECONSTITUTIONS IN *Tubifex* EMBRYOS

It was noted earlier (p. 537) that development of complete or partial twins from single eggs is not infrequent in certain oligochetes under supposedly natural conditions. Partial duplications may involve either the anterior or the posterior end or both, but in *Tubifex* these duplications originate in different ways (Penners, 1924*a*, *b*). Simple duplications of the anterior end result from failure of the germ bands of the two sides to come together anteriorly; the bands of each side give rise to an anterior end, symmetrical as regards body wall, nervous system, and entoderm but with only one dorsal and one ventral series of setae and one nephridium in each segment, seta-sacs and nephridia of the apposed sides not developing. Penners suggests that these forms result from action of some

⁵ The designations an_1 - veg_2 are the same as used for the sea-urchin embryo (p. 438).

unknown inhibiting factor which prevents normal union of germ bands anteriorly. It may be suggested further that absence of setae and nephridia on the apposed sides may result not from lack of capacity to reconstitute these organs but from mutual inhibition of the two parts, as in many cases of postembryonic reconstitution. The other form of partial twinning is a cruciate duplication, resulting from duplication of the germ bands and union of one band of each pair to form the two anterior ends (Fig. 176). This type of duplication has also been produced experimentally by subjecting eggs of *Tubifex* to high temperature together with low oxygen content of water (Penners, 1924*b*). Under these conditions the first cleavage is equal instead of unequal, as normally; and in later cleavages two *2d*- and two *4d*-cells, instead of one, appear. Equal division of the cell *CD* at the second cleavage gives the same result. Each of the *2d*-cells gives rise to a pair of ectodermal germ bands, and each of the *4d*-cells to a pair of mesodermal bands. According to Penners, duplication of the somatoblasts results from equal division of the polar plasms (p. 550) by the equal first cleavage.

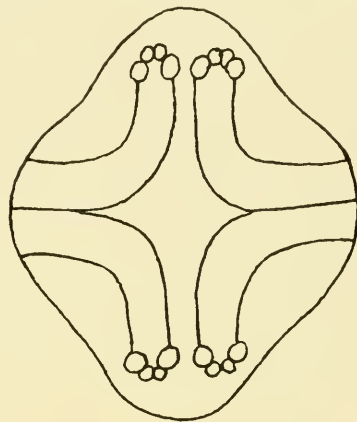


FIG. 176.—Posterior parts of ectodermal germ bands in cruciate duplication of *Tubifex* (after Penners, 1924*a*).

Development of certain blastomeres or blastomere groups of *Tubifex*, after killing others by localized ultra-violet radiation, high temperature, or strong shaking, has also been followed (Penners, 1926). The dead cells soon separate from the living. Further cleavage patterns of isolated *CD*- or *D*-cells containing the polar plasms is not altered; they give rise to the two somatoblasts, these to teloblasts and germ bands, and normal embryos result. The germ band of one side can develop and differentiate when that of the other side is killed. When the polar plasms are killed, germ bands do not develop, and the embryo consists of a compact mass of entoderm with an ectodermal cap of variable size. Ectodermal germ bands do not develop when the first somatoblast is removed, but mesodermal bands develop and differentiate. Killing of the cells *2D* or *3D*, which are in the mesoderm line, or of the mesoblast *4d* or its two daughter cells, results in absence of mesoderm. On the other hand, normally proportioned embryos develop when only part of the entoderm cells are pres-

ent. In these cases the germ bands undergo less growth, and the embryo is smaller than normal.

According to these data, the *D*-quadrant, containing the polar plasms, is potentially a whole *Tubifex* embryo, or even two embryos; but the *A*-, *B*-, and *C*-quadrants normally play a part in embryo formation. In their absence the parts normally formed by them are reconstituted by cells originating from the *D*-quadrant. Evidently, as regards this quadrant, development is not a mosaic. Penners regards the polar plasms, which are contained in the *D*-quadrant, as "organ-forming substances" and as determining the potentialities of the two somatoblasts.⁶ It is, however, an interesting question whether this conclusion, if correct, means anything more than that the polar plasms represent all the potentialities of the *Tubifex* egg cytoplasm, except perhaps those of the entoderm. What sort of formative substance can it be which gives rise to the entire ectoderm and mesoderm of an embryo or of two embryos? Are the polar plasms anything more than active, yolk-free parts of the egg cytoplasm? Do they differ from the entodermal cytoplasm except in being yolk-free? As yolk-free cytoplasmic aggregations, they may be primarily regions of more intense metabolism than other parts and consequently more or less dominant.

Many other annelids and mollusks show more or less definite aggregations of cytoplasm apically or basally or both, but their fates in cleavage differ. In some cases they are localized in the micromeres of the first quartet, and new yolk-free aggregations appear apically in the macromeres preceding formation of each quartet of micromeres. In some forms they disappear completely in early development. The case for specific significance of the polar plasms does not appear entirely convincing. Undoubtedly the cells *CD* and *D* and the somatoblasts *zd* and *qd* differ in some way from the other cells; but, since they are able to reconstitute a whole embryo if a part of the entoderm is present, the difference seems to be in the direction of less, rather than more, narrowly limited specificity.

DUPLICATIONS IN OTHER ANNELIDS AND MOLLUSKS

Duplications have been produced experimentally in the polychetes *Chaetopterus*, *Nereis*, and *Sabellaria* and in the pelecypod *Cumingia* by subjection of eggs to pressure, low temperature, high temperature, centrifuging, anaerobiosis, and KCN during a definite period preceding cleav-

⁶ See also Schleip, 1914a, b, 1929; von Parseval, 1922.

age.⁷ According to Tyler, the usual condition resulting in duplication is equal first cleavage with spindle at right angles to its normal position but in, or parallel to, the equatorial plane of the egg. In centrifuged *Chaetopterus* eggs, however, the polar lobe may be attached to the otherwise smaller cell of an unequal first cleavage, and with incorporation of the lobe into the cell the two blastomeres become equal. The two blastomeres resulting from equal cleavage have the potentialities of the *CD*-blastomere of normal cleavage, and duplications result, as in *Tubifex*. These vary in degree, some being clearly cruciate forms, the duplication involving pretrochal, as well as posttrochal, regions; others, with only posttrochal duplication. The apical organ is usually not duplicated, but eyespots, mouth, and other organs may be. In general the completeness of duplication is greater in the posttrochal, than in the pretrochal, region. Both $1/2$ blastomeres isolated following equal cleavage can develop apparently normal trochophores. The pretrochal duplications indicate that even in this region there may be some alteration in fates of some of the cells in consequence of altered relations to other cells. The wide range in degree of completeness of posttrochal duplication and the frequent development of complete and separated posttrochal regions indicate that the somatoblasts are totipotent for ectoderm and mesoderm of this region and capable of reconstitution.

ISOLATIONS AND TRANSPLANTATIONS IN ANNELID AND MOLLUSK EMBRYOS

Isolation experiments with cleavage stages of the polychete *Sabellaria* show that the formation of the apical tuft of cilia depends on the presence of the first polar lobe and the *C*-cell; development of the posttrochal region, on the presence of *D*. Transplantations and unions of blastomeres and of whole eggs show complete self-differentiation of all blastomeres. Exogastrulae produced by treatment with alkaline isotonic NaCl show completely independent development of ectoderm and entoderm with no evidence of induction. However, duplications result from KCN treatment; evidently reconstitution of pattern is possible in the region from which the somatoblasts form.⁸

After removal of the polar lobe of the two-cell stage of *Ilyanassa* the second cleavage is equal; and the cell *4d*, the mesoblast in normal development, is the same size as other cells of the fourth quartet, instead of much

⁷ Titlebaum, 1928; Tyler, 1930; Novikoff, 1939.

⁸ Novikoff, 1938, 1939, and 1940, "Morphogenetic substances and organizers in annelid development," *Jour. Exp. Zool.*, 85. See also Hatt, 1931, 1932.

larger, as normally, and the resulting larvae develop a prototroch but die before attaining the veliger stage. In general, isolated blastomeres cleave as if other blastomeres were present, and may develop into defective forms with or without cilia but with entoderm overgrown by ectoderm (Crampton, 1896).

The studies by E. B. Wilson (1904) give additional information on development of isolated parts of eggs and early stages of the mollusks *Dentalium* and *Patella*. In the living, unfertilized *Dentalium* egg two white polar areas are visible, the intervening region being superficially pigmented: the basal white area enters the polar lobe when that forms. Apical pieces of the unfertilized egg, when fertilized, do not form a polar lobe; the first two cleavages are equal; and ciliated swimming larvae may develop but almost always lack the apical tuft of long cilia. The basal piece, when fertilized, forms a polar lobe, cleaves like the entire egg, and may develop into a normal trochophore. Pieces isolated by apicobasal section through the basal white area may cleave like whole eggs and give rise to "nearly normal" trochophores. The apical piece of the fertilized egg develops like that of the unfertilized; but the basal, nonnucleated piece forms the three polar lobes successively without dividing. After removal of the polar lobe in the two-cell stage, the following cleavages are essentially equal, and *2d* and *4d* are no larger than other cells; and in the resulting larvae the posttrochal region is absent or small and rounded and never develops further, and the apical tuft is also absent. If the first polar lobe is allowed to complete its normal cycle with return to the cell body and the second lobe is removed, the resulting larvae also lack the posttrochal region, but the apical tuft is present. Larvae from the *AB*-blastomere of the two-cell stage or from *A*, *B*, or *C* of the four-cell stage are similar, except in size, to those from which the first lobe has been removed; but larvae from the *CD*- or the *D*-blastomere, of which the polar lobe is a part, possess both posttrochal region, usually "too large," and apical tuft. Wilson concludes that the material of the lobe must be specific and the determining cause of development of apical organ and posttrochal region. Centrifuge experiments indicate, however, that the polar lobe of another mollusk, *Ilyanassa*, represents activity of the cortex which is not displaced by centrifuging and that altered distribution in the lobe of other cytoplasmic constituents has no effect on its formation or on further development. Effects of hydrostatic pressure in inhibiting lobe formation and cleavage point to the same conclusion.⁹ If the lobe is cortical, the relation between the first

⁹ Morgan, 1935, 1936; Pease, 1940. See also F. R. Lillie, 1906.

lobe and the apical tuft probably results from an inhibition of development following removal of the lobe rather than from presence in the lobe of a specific tuft-forming substance. In any case, effects of removal of lobe indicate that development in the forms concerned is not a mosaic. The relation of lobe or somatoblast and apical tuft suggests the possibility that the first somatoblast may have some inducing action. Novikoff (1940, see footnote 8, p. 559) maintains that a cytoplasmic substance present in the polar lobe of *Sabellaria* directs development of the cell or cells into which it enters and is to be regarded as both a morphogenetic substance and an organizer.

The first two cleavages of *Patella* are almost or quite equal; consequently, the *D*-quadrant cannot be distinguished in early stages, and *zd*, supposedly the first somatoblast, is no larger than other cells of the second quartet. Isolated blastomeres cleave as if the other cells were present; but partial forms, even those from 1/8 and 1/16 blastomeres, usually form closed embryos and may gastrulate if entoblast cells are present. Isolated 1/4 blastomeres and isolated micromeres of the first quartet from all four quadrants give rise to larvae with apical tuft, differing in this respect from *Dentalium*, in which only the *D*-quadrant develops the tuft and only if the first polar lobe is not removed. Isolated cells of the first quartet develop according to their lineage into cells of the apical organ, ciliated prototroch cells, and other ectoderm cells. Apparently dorsiventral pattern is further advanced in development at beginning of cleavage in *Dentalium* than in *Patella*.

GENERAL SUGGESTIONS CONCERNING SPIRAL CLEAVAGE

The very general decrease basipetally in rate of cleavage in early stages suggests an apicobasal gradient of some sort, and other evidences of such a gradient have been found (pp. 119, 545). The fixity or stability of pattern apparently undergoes some decrease basipetally. The regions of most stable determination or specific constitution, as indicated by the isolation experiments, represent apical or anterior regions of the larva and are essentially head regions. In this respect the forms with spiral cleavage resemble many other forms. Isolated apical regions of adult hydroids and heads of planarians and annelids do not reconstitute other parts, but apical regions or heads can reconstitute from lower levels. Essentially similar conditions appear in sea-urchin embryos.

At some time in annelid development a ventrodorsal gradient must appear, at least in the posttrochal region, for such a gradient is characteris-

tic of the adult polychete and oligochete. In the polychete this gradient may be initiated by the coming-together ventrally of the active borders of the somatic plate and in the oligochetes by the apposition ventrally of the germ bands.

The highly stable condition of the more apical regions in early stages and the high capacity for reconstitution of the adults of many annelids has sometimes been regarded as presenting a puzzling problem. However, the most stably determined cells of the apical region, such as the trochoblasts and apical tuft cells, apparently take no part in postlarval development, and even in the adult the head region is apparently fixedly determined. But in the embryo the *D*-quadrant and the somatoblasts derived from it are totipotent for ectoderm and mesoderm and are capable of reconstitution. They are no more determined or differentiated than post-cephalic regions in the adult, perhaps less so.

The most striking features of spiral cleavage are the definiteness of cleavage pattern in a given species and the differences of pattern in rather closely related species. However, cleavage pattern can be altered in various ways, as will appear in following sections. The cleavage pattern is evidently an expression of a cortical or general cytoplasmic pattern of some sort in the egg, but the oblique form of cleavage apparently masks to some extent the organismic pattern in early stages, so that it appears only gradually and does not at first correspond in different cell groups. Apparently the factors that determine cleavage planes oblique to the polar axis are not very closely associated with organismic developmental pattern, but differences in cell size are probably related in some way to this pattern. The gradual appearance of bilateral pattern, the lack of correspondence in the bilateralities of different regions in earlier stages—for example, in the two somatoblasts and their derivatives—and the gradual “adjustment” in the course of development are difficult to account for in terms of a developmental mosaic. The fact that isolated cells or cell groups are capable of more or less differentiation in the same way as if other parts were present does not prove that they are independent of other parts in the intact organism.

Although spiral cleavage pattern is relatively stable in those groups in which it appears, the occurrence of widely different cleavage patterns in related groups seems not without significance, as indicating that cleavage pattern and organismic pattern are not very closely related. Among the turbellaria spiral cleavage appears only in polyclads. Cleavage of the acaeolous *Polychaerus* differs from the spiral type, according to Gardiner, and

cleavage of rhabdocoels and triclads is apparently not spiral; also, trematodes and cestodes seem not to have spiral cleavage. Rotifer cleavage pattern is apparently spiral in early stages but later departs from spiral pattern (Zelinka, 1891; Jennings, 1896). If the rotifers are related to ancestral forms of the "Trochelminthes" and if the spiral cleavage pattern has ancestral significance, we might expect to find it in well-developed form in the rotifers. Cleavage in cephalopods is meroblastic and widely different from the spiral type (Vialleton, 1888; Watase, 1891). Spiral cleavage may appear to be closely associated with early differentiation of free-swimming larvae of trochophore type, but in oligochetes and leeches and in fresh-water pelecypods there are no such larvae, but cleavage is spiral. Also, the pilidium larva of nemerteans is very different from the trochophore type, and some nemerteans develop without pilidium larvae; but spiral cleavage is present in both.¹⁰

Whatever conclusions we may draw concerning the phylogeny of groups with spiral cleavage and their relatives without, it seems probable that we must distinguish between the oblique, or so-called "spiral," type of cleavage, as determined by physiological factors not closely associated with organismic developmental pattern, and the relative sizes of cells and their differences in different groups, as expressions of that pattern. The oblique form of cleavage corresponds closely to a surface-tension pattern, but relative cell sizes suggest regional differences in cortical activity or differences in the deeper cytoplasm.

CLEAVAGE AND RECONSTITUTION IN CTENOPHORE DEVELOPMENT

Pattern of cleavage and early development of ctenophores differs from those of other groups, and present knowledge serves chiefly to make evident the lack of any real insight into its physiology.¹¹ In the undivided egg of *Pleurobrachia* a thick ectoplasm or cortical layer, uniformly distributed about the egg, stains yellow with neutral red and rose with Nile blue sulphate, as if alkaline in reaction, in sharp distinction from the interior, which stains red or blue. The egg of *Beroë* shows no such differential staining, but with dark-field illumination a clearly defined green ectoplasm or cortex is visible (Spek, 1926). Polar bodies form at what is considered to be the basal or vegetal pole, that is, the later oral pole; and the

¹⁰ Spiral cleavage has been followed through the earlier stages in a fresh-water nemertean *Stichostemma*, with direct development (Child, unpublished).

¹¹ Chun, 1880, 1892; Driesch und Morgan, 1895; Fischel, 1897, 1898, 1903; Ziegler, 1898, 1903; Rhumbler, 1899; Yatsu, 1911, 1912a, b; Spek, 1926.

nucleus remains near this pole. First and second cleavages are meridional and equal, the cleavage furrows gradually progressing from the basal pole (Fig. 177, *A*). The two cleavage planes correspond, respectively, to the stomodeal or esophageal and the tentacular plane of the adult. The third cleavages are somewhat oblique in opposite directions on each side of the esophageal plane, so that four slightly smaller macromeres lie somewhat apical to four larger ones (Fig. 177, *B*). Each of the eight cells then gives

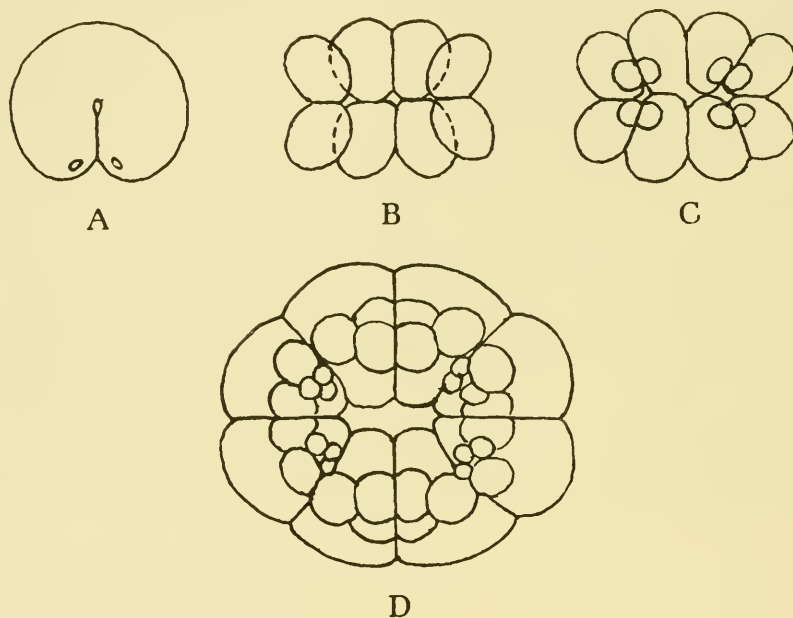


FIG. 177, *A-D*.—Early cleavages of ctenophore egg. *A*, the first cleavage furrow passing through egg, nuclei near basal pole; *B*, eight-cell stage; *C*, sixteen-cell stage; *D*, later stage on larger scale (after Ziegler, 1898).

rise to a micromere from its apical region (Fig. 177, *C*); these micromeres divide, and other generations of micromeres form (Fig. 177, *D*), resulting in four more or less separated groups of micromeres, corresponding to the four quadrants. According to Spek's observations with dark-field illumination, the green ectoplasm of the *Beroë* egg aggregates about the basal region before each of the first three cleavages, and the cleavage furrow begins there. The green ectoplasm forms the head of the cleavage furrow and a layer on each side as cleavage progresses apically and, following first and second cleavages, is again uniformly distributed over the surfaces of the cells. Following the third cleavage, however, the green ectoplasm is local-

ized in the apical regions of the macromeres, and the micromeres are formed from it. Spek holds that this fixed localization results from a marked increase in cytoplasmic viscosity following the third cleavage. His observations lead him to the conclusion that the egg is not a mosaic, though different substances are undoubtedly present in it. It seems evident that this orderly series of events in early cleavages does not indicate independence of parts. The mosaic condition is brought about, according to Spek, dynamically during cleavage through the activities associated with mitosis and the changes in viscosity. To what extent the ectoplasmic changes result from actual migration of the ectoplasm, as distinguished from regional separation of cytoplasm proper from yolk or other inclusions, in consequence of altered physical, associated with metabolic, conditions, is perhaps still a question. Is the ectoplasm associated with the cleavage furrow, or is that finally localized in the apical regions of the macromeres, necessarily the same ectoplasm that originally extended over the egg surface?

The four groups of apical micromeres spread over the macromeres and give rise to the apical nervous system and sense organ, the swimming-plate rows, two rows from the cells of each group, general ectoderm, and stomodeum. The eight macromeres divide into sixteen entoderm cells, and each gives off a micromere basally which contains the last traces of the green ectoplasm (Spek) but becomes entoderm. Later an entodermal pouch develops in each quadrant and divides into two canals, each leading to a plate row.

There is no directly visible evidence of polarity in the cytoplasm of the undivided egg, but the relation of the growing oöcyte to the parent body (Chun, 1880) suggests the possible determination of polarity by the differential resulting from this relation. On the other hand, it is possible that position of nucleus near the egg surface is not definitely predetermined but is a matter of chance, and that polar-body formation there constitutes a step in development of polarity, the orderly behavior of ectoplasm in relation to this region another step, and its apical localization the final stage in establishment of polar pattern. That the bisymmetrical pattern is also determined dynamically in relation to the ectoplasmic activities associated with first and second cleavages seems not improbable.

Cleavage and development of isolated parts of the ctenophore egg, blastomeres, and blastomere groups have been studied by a number of investigators.¹² In isolated parts of undivided eggs relation of plane of sec-

¹² References given in footnote 11, p. 563.

tion to polar axis is not certainly known or can be inferred only from developmental results, but in eggs beginning first cleavage and later stages definite orientation of plane of section is possible. After removal of an apical portion by section more or less transverse to the apicobasal axis, development differs according to amount and region removed. Removal of parts by oblique section basal to the equator usually results in larvae with one or more plate rows defective or lacking; but after removal of parts by section apical to the equator, normal larvae may develop. After lateral removal by section parallel to the apicobasal axis one or more plate rows are usually defective or absent. Defects are greater after formation of polar bodies than before (Yatsu). From the experimental data Fischel concluded that plate-row material is localized in a ring near the basal pole, but Spek regards regional differentiation of the ectoplasm before or at beginning of first cleavage as improbable and maintains that defective development or absence of one or more plate rows results from removal of a certain amount, rather than a particular kind, of ectoplasm. Since the ectoplasm aggregates basally before the first cleavage, removal by section of cytoplasm about this region will remove a relatively large amount of ectoplasm and so interfere later with development of some of the micromeres. The defective development of one or more plate rows following removal of peripheral cytoplasm basal to the equator seems to indicate that the ectoplasm does actually migrate apically, as Spek asserts, for the plate rows develop about the apical region.

Cleavages of isolated blastomeres, as far as followed, occur always as if the other cells were present. Each $1/2$ blastomere gives rise to four plate rows, each $1/4$ blastomere to two, each $1/8$ to one. According to Yatsu, the end cells and the middle cells of the eight-cell state (Fig. 177, *B*) apparently differ in some way, for in groups of middle blastomeres the number of plate rows developing is often less than the number of middle cells present; but isolated single or paired end cells give rise, respectively, to one or two rows. In groups including both middle and end cells the number of plate rows developing is sometimes greater than the number of $1/8$ blastomeres present, suggesting some reconstitution. Groups of $1/16$ blastomeres, including more of the micromeres than of the macromeres, usually develop plate rows equal in number to the micromeres, but sometimes fewer (Fischel, Yatsu).

The ectoderm of these partial forms incloses the entoderm completely; but the side of the larva representing the original surface of contact with other cells remains flattened, and the stomodeum invaginates from the

basal region of this side. The number of entodermal pouches is very commonly greater than the number of macromeres present, that is, a $1/2$ form has three, a $1/4$ form two, the extra pouch being smaller than the others. Driesch and Morgan regarded this as a reconstitution, but Fischel holds that the extra pouch results from mechanical division of entoderm by the invaginating stomodeum.

Displacement of micromeres into two separate groups results in larvae with two apical organs and four plate rows about each; but, although such displacement almost always involves more or less displacement of macromeres, the larvae are single as regards entoderm and stomodeum. Isolation experiments agree in general in showing that plate-row-forming cells or cell groups are so far determined at an early stage that isolation does not usually alter their course of development. The most interesting point is the usual absence of reconstitution of plate rows in the partial forms. The adult ctenophore shows high capacity for reconstitution of plate rows from general ectoderm, as well as of other parts;¹³ consequently, the general ectoderm must be potentially capable of giving rise to plate rows. Perhaps developing plate rows exercise some degree of dominance over the general ectoderm, and this may be adequate in the small larval forms to inhibit reconstitution of other rows. Since the plate rows are undoubtedly regions of more intense physiological activity than the general ectoderm, they probably influence it in some way. With increase in size of the individual this dominance may not extend over the whole ectoderm between rows, and reconstitution of other rows becomes possible. The fact that partial forms sometimes develop more, sometimes fewer, plate rows than expected from the number of micromere groups present is of interest in this connection, as suggesting such relations. Also of interest are the observations of Chun (1892, 1895) on $1/2$ larvae of *Bolina* found after a storm which had presumably isolated blastomeres. These forms had developed to larvae as $1/2$ forms, but on metamorphosis they reconstituted to wholes.

Except as regards the plate rows, the forms resulting from isolation and displacement of blastomeres show considerable reconstitution. Duplication of apical organs and arrangement of plate rows about the duplicated organs involves change in position and direction of some of the plate rows, if not actual reconstitution. In $1/2$, $1/4$, and $1/8$ forms ectoderm overgrows the entoderm completely; that this is entirely a mechanical matter seems doubtful. Also, the stomodeum in such forms is a whole, not a par-

¹³ Mortensen, 1913; Coonfield, 1936a, b, 1937a, b.

tial, stomodeum. That the extra entodermal pouch is always the result of mechanical division of the entoderm by the stomodeum, rather than a true reconstitution, may be questioned. And, finally, reconstitution in early stages may be limited by viscosity of the cytoplasm. The fact that forms developing from isolated blastomeres remain flattened on the side formerly in contact with other cells indicates a considerable physical rigidity. Spek finds a marked increase in viscosity at the third cleavage. Physical rigidity, as well as regional specificity, may limit reconstitution.

Evidently the undivided ctenophore egg is not a mosaic but a dynamic system in which definite orderly and related changes take place. From the data at hand it appears possible that before polar-body formation the cytoplasm has no definite organization except a surface-interior difference and that axiate pattern develops gradually. The ectoplasmic activities of first and second cleavage may be factors in determining the biradial pattern. In short, it seems possible that the polar-biradial pattern of the ctenophore can originate without any further "organization" than the surface-interior, ecto-entoplasmic pattern, the eccentric position of the nucleus, and the ectoplasmic activities during the first and second cleavages. Since the plate row of the adult and the general ectoderm show a very definite apicobasal gradient (p. 106) and the apical region is dominant in reconstitution (Coonfield, 1936a), gradient pattern must develop in the descendants of the micromeres at some stage.

CLEAVAGE PATTERN AND DEVELOPMENT OF *Ascaris*

Cleavage pattern, cell lineage, and the "germ path" of *Ascaris megalocephala* (= *equorum*) have been repeatedly and extensively studied.¹⁴ The germ path, that is, the persistence of a small number of large chromosomes in the blastomere line from which the germ cells develop, and the diminution of chromatin by exclusion from the nucleus of the terminal portions of the large chromosomes and appearance of a larger number of small chromosomes in other cells, has been regarded as a feature of particular interest (Fig. 178). Essentially similar cleavage patterns have been observed in various other nematodes, but chromatin diminution apparently does not occur in all.

The egg and embryonic stages of *Ascaris*, inclosed, as they are, in a hard thick shell, are less directly accessible to some experimental procedures than those of many other forms; but blastomeres have been killed

¹⁴ Boveri, 1887, 1890, 1892, 1899, 1910b; C. C. Schneider, 1891; Herla, 1893; Zur Strassen, 1896; Zoja, 1896; H. Müller, 1903; Bonfig, 1925; *et al.*

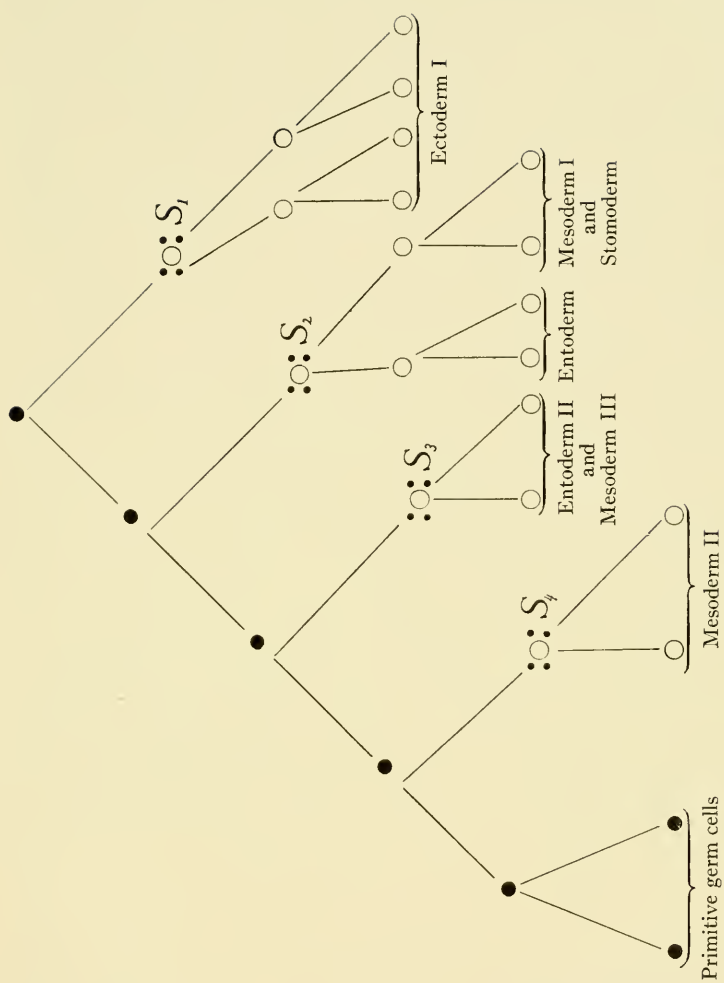


FIG. 178.—Diagram of germ path and cell lineage in early development of *Ascaris megalocephala*. Black circles represent cells before chromatin diminution and primitive germ cells, which do not undergo diminution; unshaded circles with four black dots about them represent cells undergoing diminution and unshaded circles alone, the cells after diminution; further history of cells is indicated (after Boveri, 1910b).

by localized radiation, and pressure and centrifuging have been employed in experimental analysis. The ovarian oöcyte is more or less pear-shaped and attached to the rhachis of the gonad by its slender end. Supposedly

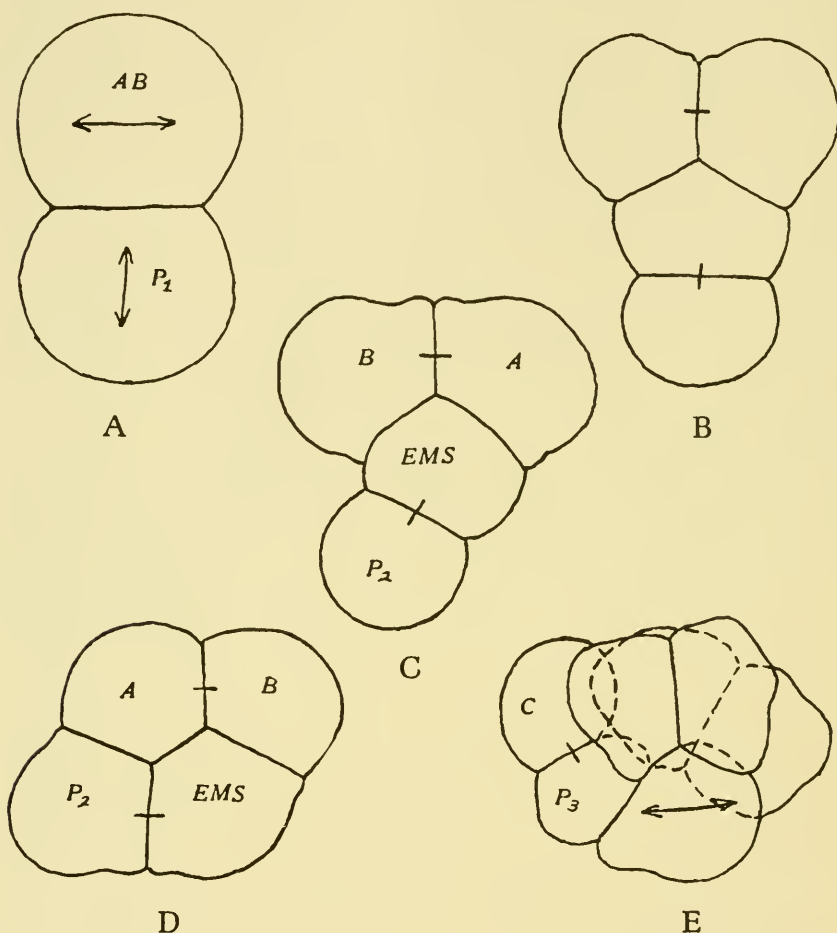


FIG. 179, A-E.—Early cleavages of *Ascaris megalocephala*. A, two-cell stage, spindles of second cleavages indicated; B, four-cell stage; C, the change in cell position to form the "rhombus"; D, four-cell stage after change in position, the rhombus; E, seven cells, showing asymmetry in position of the four dorsal cells. In the figures dorsal is above; anterior, right (after Boveri, 1910b).

the blunt free end becomes the apical pole. Normally the egg becomes spherical before polar-body formation; but in eggs which retained the elongated form until fertilization and shell formation, polar bodies were

observed to form at the blunt pole in about three-fourths, elsewhere in others. Most of these eggs developed abnormally, but in 91 per cent of the normal cleavage stages polar bodies were at the blunt pole (Schleip, 1924). After fertilization and maturation the yolk becomes more densely aggregated basally, so that an apicobasal gradient is visible in the cytoplasm.

The cleavage pattern of *Ascaris* and of various other nematodes studied differs from other known patterns. The first cleavage is transverse to the apicobasal axis and is practically equatorial (Fig. 179, *A*), the apical cell, *AB*, being regarded as dorsal, the basal cell, *P*₁, as ventral; the two cells are different, *P*₁ containing more yolk. The second cleavage is meridional in relation to the egg axis and is accompanied by diminution of chromatin in the dorsal *AB*, transverse and without diminution of chromatin in the ventral *P*₁ (Fig. 179, *B*). A change in position of blastomeres follows this cleavage (Fig. 179, *C*), resulting in a "rhombus" form (Fig. 179, *D*). Up to this stage it has not been possible to distinguish future anterior and posterior ends; but in the rhombus *P*₂ and the cell with which it comes into contact (*B*) are posterior, the other two cells anterior, and the nuclei of all four cells are in the median plane. According to Boveri, *A* and *B* give rise to general ectoderm, largely of the anterior and dorsal regions; *EMS* forms entoderm, mesoderm, and stomodeum; and *P*₂, ectoderm, mesoderm, and germ cells. Evidently there is no segregation of embryonic layers except entoderm in particular cells. At the next cleavage *A* and *B* divide equally in the median plane; but the four resulting cells shift to somewhat oblique positions, so that in lateral view the right anterior cell of the four is higher than the left anterior, producing a slight asymmetry (Fig. 179, *E*). The cell *EMS* divides into posterior (entoderm) and anterior (mesoderm and stomodeum) cells, both of which undergo chromatin diminution. The spindle of this division is indicated in Figure 179, *E*. *P*₂ also divides into *C* and *P*₃ without diminution, but at the next division *C* undergoes diminution, giving rise later to ectoderm.

With change in position of cells of the four-cell stage in a direction at right angle to the usual direction, a tetrahedral form (Fig. 180, *A*), instead of a rhombus, results. Intermediate directions of the shift occur, but finally result in either rhombus or tetrahedral form. The tetrahedral form supposedly results from injurious or inhibiting conditions; high temperature increases its frequency (Bonfig, 1925). In this form the two dorsal cells divide transverse to the axis of the two ventral cells, that is, at right angles to their divisions in the rhombus form; and the cell on the left side and anterior with respect to the ventral cells is higher in lateral view (Fig.

180, *B*), instead of the right anterior cell, as in rhombus development (Fig. 179, *E*), a reversal of the asymmetry of the dorsal cell group. The adult *Ascaris* is asymmetrical in that the nucleus of the unicellular excretory organ is usually on the left side, but in one individual to about thirty or forty it is on the right, and the proportion of reversed asymmetry in cleavage is about the same; consequently, it is concluded that the asymmetry of cleavage determines that of the adult (Zur Strassen, 1896).

In spite of the numerous studies on *Ascaris* development, the relation of egg and cleavage pattern to axiate organismic pattern is still obscure. Apparently egg polarity does not coincide with the longitudinal axis of the animal. Granting this, how is the anteroposterior axis determined? The two dorsal cells, *A* and *B*, of the four-cell stage are not distinguishable as

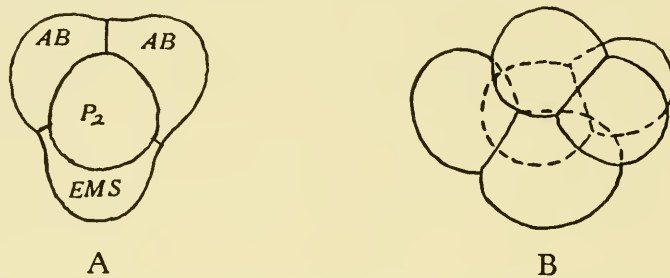


FIG. 180.—*A*, tetrahedral four-cell stage; *B*, reversed asymmetry of dorsal cells resulting from tetrahedral form (after Bonfig, 1925).

anterior and posterior until the change in position results in the rhombus form. Then the cell in contact with *P*₁ is distinguishable as posterior (*B* of Fig. 179, *D*) from *A*, the anterior cell. But the relative change in position of dorsal and ventral cells is not always in the same direction; it may be at right angles to the usual direction, producing the tetrahedral form, and the two dorsal cells are right and left, instead of anterior and posterior, in relation to the ventral cells and apparently also in relation to the anteroposterior axis of the animal. Is there normally a physiological difference between the two dorsal cells which determines the direction of shift in position in the four-cell stage but which is more or less completely obliterated in slightly inhibiting conditions? If this is the case, how are anterior and posterior end determined in the tetrahedral forms? Or are the two dorsal cells alike, and does the shift occur indifferently in either direction to form the rhombus and at right angles to these directions to form the tetrahedron? If this is the case, the ventral cells apparently determine the anteroposterior axis.

Although definite answers to these questions seem to be lacking, certain features of *Ascaris* development are suggestive. There is an apicobasal gradient, indicated by differential aggregation of yolk in the undivided egg. From his study of dispermic eggs Boveri (1910b) concluded that the polarity of the egg is represented by a cytoplasmic gradient and that nuclei coming to lie in the apical levels of this gradient undergo diminution, while those in the basal region do not. The behavior of dorsal and ventral cells in normal cleavage suggests that the gradient differential is greater in the ventral (basal) than in the dorsal (apical) half of the egg. The dorsal cell, *AB*, of the two-cell stage undergoes diminution as it divides into *A* and *B*; the ventral cell does not. However, at the next division the cell, *E*, evidently the more apical part of the basal half, undergoes diminution; but the cell, *P*₂ representing the extreme basal region, does not. Evidently there is sufficient gradient in the ventral (basal) half of the egg to determine this difference in nuclear behavior. Moreover, the dorsal cells, *A* and *B*, form only part of the general ectoderm, while most of the organs develop from the two ventral cells. The apical half of the egg probably represents the higher levels of the apicobasal gradient, but with little axial differential, most of the differential being in the basal half. The apicobasal axis of the sea-urchin egg is apparently somewhat similar. If these suggestions are correct, the ventral cells of the four-cell stage may determine the longitudinal axis.

In formation of the rhombus some unknown factor determines the change in position of the cells, at least as regards plane of the shift. In tetrahedral forms this factor has apparently been somewhat less effective. The factors determining the asymmetry in position of the four dorsal cells from the six-cell stage (Fig. 179, *E*), its reversal in tetrahedral forms (Fig. 180, *B*), and the postulated relation between this asymmetry and that of the adult, which concerns a single cell, are also completely unknown.

Cleavage and development of blastomeres of two-cell and four-cell stages, after killing or preventing development of others by localized radiation, are stated always to be essentially as if the other cell or cells were also developing (Stevens, 1909). The presence of the dead or inhibited cells apparently does not determine the partial character of development, since contact between them and the living cells is often slight. There is no conclusive evidence of reconstitution.

Eggs kept for several months without exposure to air show various evidences of injury in their development on exposure to air. In some all chromosomes pass into one cell at the first cleavage, diminution may take place at the next division, and a blastula-like cell mass is formed, as with

an isolated *AB*-cell, or the cell containing all chromosomes may develop like a whole egg (Fauré-Fremiet, 1913). It seems probable that these differences in development result from alterations in the apicobasal gradient pattern by the inhibiting conditions and the following recovery. Eggs allowed to develop by exposure to air after some 3 months in CO_2 give forms ranging from normal to unorganized cell masses, but a third of them show various degrees of what appears to be a differential inhibition, more or less of the anterior region being present merely as an unorganized cell mass and posterior regions normally developed (Painter, 1915).

Sometimes a considerable part of the cytoplasm, usually containing the nucleus of the second polar body, constricts off from the apical region but does not divide. The remaining part of the egg divides into a dorsal and a ventral cell, and these change their position with respect to this *Nebenzelle* in the same way as the two ventral cells of the normal four-cell stage. The following cleavages may be normal or more or less altered by presence of the *Nebenzelle*; but, with a single exception, normal animals have not been seen to develop from these eggs (Kautsch, 1912).

Although an apicobasal gradient pattern is indicated in the fertilized *Ascaris* egg, it does not necessarily follow that regional cytoplasmic differences are merely quantitative, but conclusive evidence for regional specificity at the beginning of development is lacking. The undivided egg appears not to be a mosaic, and the change in position of cells at the four-cell stage suggests physiological relations of some sort between the blastomeres. As regards cleavage pattern, regional characteristics are apparently determined early, but independent differentiation of isolated blastomeres does not proceed very far. If the ventral cells of the four-cell stage do determine the anteroposterior axis, they perhaps induce some degree of anteroposterior differential in the dorsal cells. It is evident, however, that the cleavage pattern of *Ascaris* gives us little definite information concerning developmental pattern.

ENTOMOSTRACAN CLEAVAGE PATTERN

The normal cleavage pattern of *Cyclops* is determinate, at least as regards mesoderm, endoderm, and primitive germ cells. The germ path is indicated by certain cytoplasmic granules with a definite cell lineage from the first cleavage to the primitive germ cells. The plane of first cleavage is said to be in the shortest diameter of eggs deformed by pressure in the egg sac; and, according to Fuchs (1914) and M. Jacobs (1925), the cleavage axis has a definite and constant relation to developmental pattern and be-

comes the dorsiventral axis of the embryo but is independent of the axis indicated by polar-body position. If these observations are correct, organismic pattern appears to be determined by cleavage pattern or by the same factor that determines cleavage pattern; and if a polarity is determined in the ovary or by position of polar body-formation, it may be obliterated by the polarity of the cleavage pattern, which is, or may be, determined by pressure. Development of centrifuged eggs seems to support this conclusion (p. 586). There is, however, no evidence of a polar differential in earlier cleavage. The first two cleavages divide the egg into four almost equal quadrants, with polar cross-furrows, as in spiral cleavages, determining a cleavage axis (Fig. 181, *A*), which supposedly becomes the

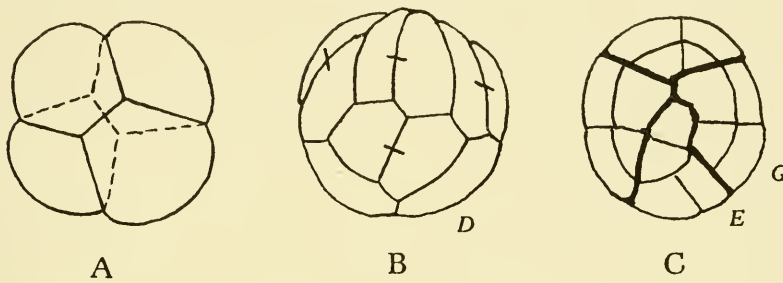


FIG. 181, *A-C*.—Cleavage stages of *Cyclops*. *A*, four-cell stage; *B*, sixteen-cell stage; *C*, later stage, showing entoderm cell, *E*, and primitive germ cell, *G*, which are formed by division of *D^{iv}1* of sixteen-cell stage shown in *B*; heavy line in *C* indicates boundaries of quadrants of four-cell stage (after Fuchs, 1914).

dorsiventral axis of the animal, but dorsal and ventral are distinguishable only in later stages. The first cleavage plane is said to coincide approximately with the median plane of the embryo. The third cleavage is equatorial with respect to the cleavage axis determined by the first two cleavages and is approximately equal; the fourth is meridional or apparently slightly oblique (Fig. 181, *B*). Entoderm develops from a single cell of the fifth cleavage generation, and its sister cell is the primitive germ cell; both lie at the ventral pole and approximately in the future median plane (*E*, *G*, of Fig. 181, *C*); and cells immediately surrounding them give rise to mesoderm, which is invaginated with entoderm and germ cell. This cell lineage gives no information as to origin or character of organismic developmental pattern. If it is true that cleavage pattern is determined by shape of the egg and that the cleavage axis becomes the dorsiventral axis, it apparently follows that axiate organismic pattern is independent of any pre-existing pattern or egg organization and originates from purely for-

tuitous external conditions. But how cleavage pattern is determined in a spherical undeformed egg does not appear. If the cleavage axis is the dorsoventral axis of the animal, the origin of the anteroposterior axis remains obscure. The only evidence of a differential in the accounts of cleavage is the somewhat less rapid division of the quadrant from which entoderm and primitive germ cell originate. When blastomeres are killed or inhibited by localized ultra-violet radiation but not removed, development of remaining blastomeres is not altered, except for more or less overgrowth of the undivided cell or cells by ectoderm (M. Jacobs, 1925). These experiments give further evidence concerning relation of embryonic axes to cleavage pattern, confirming and extending that from normal development. In *Cyclops* and also in *Polyphemus* the germ path may be either in a right or in a left quadrant, that is, asymmetry of cleavage pattern in this respect may be either dextral or sinistral.¹⁵

In eggs of various other entomostracans studied a cytoplasmic polarity is distinguishable, either before or after maturation, and becomes the axis of differentiation; or this axis coincides with the axis indicated by polar-body position.¹⁶ In *Polyphemus*, however, first and second cleavages are oblique to the egg axis, but this axis becomes the axis of differentiation (Kühn, 1912); but in *Lepas*, according to Bigelow (1902), there is a shift of the egg axis in relation to the oblique first cleavage. According to these data, there are considerable differences in cleavage pattern and in its relations to egg pattern and to pattern of differentiation among the entomostraca; some of the conclusions suggest that further investigation is highly desirable.

ASCIDIAN CLEAVAGE AND DEVELOPMENTAL PATTERN

Cleavage pattern and developmental pattern of ascidians are apparently closely related. In the freshly extruded egg of *Styela* (= *Cynthia*) polarity is indicated only by position of the nucleus close to the egg surface, the region in which it lies becoming the apical pole. Following extrusion the nucleus breaks down, an apical area of clear cytoplasm appears, and the first polar spindle forms in it. The egg also shows a surface-interior pattern, an ectoplasm containing yellow pigment, and an entoplasm with yolk (Conklin, 1905a). Whether the nuclear position near the surface is determined by a pre-existing cytoplasmic polarity or is itself the determin-

¹⁵ Kühn, 1912; Fuchs, 1914; M. Jacobs, 1925.

¹⁶ Grobben, 1879, 1881; Samassa, 1893; McClendon, 1906, 1907; Müller-Calé, 1913; Kruger, 1922.

ing factor of polarity is apparently not known. Sperm entrance in a region about the basal pole suggests presence of a cytoplasmic pattern (cf. Dalcq, 1932c, 1935). Evidence of cytoplasmic dorsiventrality or bilaterality in the unfertilized ascidian egg has been presented by van Beneden and Julin (1884) and by Dalcq and Vandebroek (1937).

Following sperm entrance, an extensive and remarkable series of cytoplasmic movements takes place in *Styela* (Conklin, 1905a). The yellow ectoplasm first streams basipetally and aggregates in the region about the sperm nucleus. Dalcq and Vandebroek (1937) find that local vital staining indicates movement away from the apical pole of the egg cortex about this pole in *Ascidella* (see also Vandebroek, 1936b). The sperm nucleus migrates to a position near the egg equator, but on one side of the egg axis; and the yellow cytoplasm goes with it and finally forms a yellow crescent transverse to the polar axis and symmetrical to the position of the sperm nucleus (Fig. 182, A).¹⁷ This crescent, designated as "mesoplasm" or "myoplasm," gives rise to muscle tissue. The indophenol blue, benzdinin, and leucomethylene blue tests for oxidases and peroxidases show no localization in the unfertilized egg, but, after fertilization, localization in the myoplasm (Ries, 1939; Reverberi e Pitotti, 1939). The path of the sperm nucleus and formation of the crescent suggest a dorsiventral pattern as a determining factor. Six organ-forming regions, indicated by differences in the cytoplasm, are distinguished by Conklin.¹⁸

Cleavage is, to a high degree, determinate. The plane of the equal first cleavage coincides with the median plane and divides the yellow crescent equally; the second cleavage is vertical to it and also meridional; the third separates smaller apical from larger basal cells and is slightly oblique bilaterally (Fig. 182, B). Later cleavages, so far as followed, are strictly bilateral, and the cell lineage of organs and tissues is definite (Fig. 182, C, D, E).¹⁹

The study by Chabry of development of isolated blastomeres and blastomere groups showed that cleavage and development were in all cases partial and gave little or no evidence of reconstitution, except that ectoderm formed a complete surface layer. Driesch (1895) maintained, how-

¹⁷ The side of the egg on which the crescent lies is regarded by Conklin as posterior, but comparison with amphibian development suggests that it is perhaps more nearly ventral or posteroventral, since neural plate and notochord develop on the opposite side.

¹⁸ Conklin, 1905a, 1911. See also Duesberg, 1913.

¹⁹ For earlier studies of ascidian cleavage see van Beneden et Julin, 1884; Chabry, 1887; W. E. Castle, 1892.

ever, that isolated $1/2$ blastomeres gave rise to complete or only slightly defective larvae. Cleavage of isolated blastomeres as halves was recog-

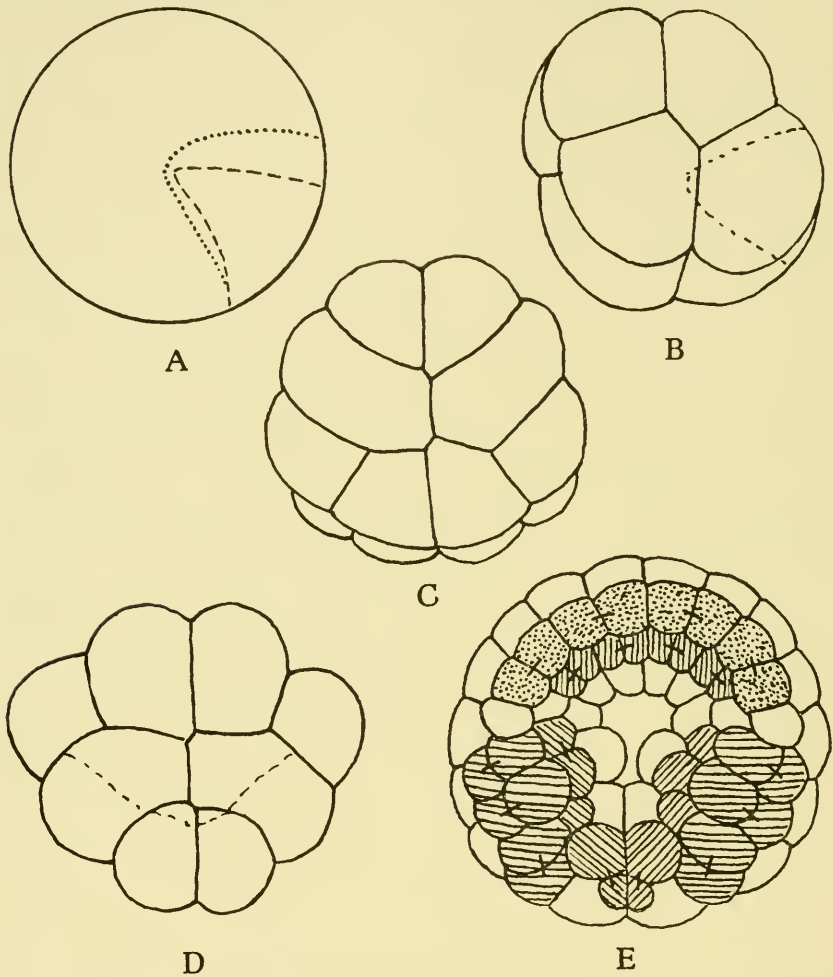


FIG. 182, A-E.—Cleavage stages of *Styela partita*. A, fertilized, undivided egg, lateral view; yellow crescent indicated by broken line; clear protoplasm at its borders, by dotted line. B, eight-cell stage in lateral view; yellow crescent indicated. C, apical, D, basal, view of sixteen-cell stage; boundary of yellow crescent indicated. E, early gastrula; neural plate cells indicated by dotting; notochord, by vertical lines; mesenchyme, by oblique lines; muscles, by horizontal lines; partially invaginated entoderm and general ectoderm, white (after Conklin, 1905a).

nized by Crampton (1897), but he also believed that whole larvae resulted. Chabry was later confirmed by Conklin,²⁰ who followed the cell lineage of

²⁰ Conklin, 1905c, 1906, 1911; experiments on *Styela partita*, *Ciona intestinalis*, *Molgula manhattensis*, and *Phallusia mammillata*.

partial forms and concluded that an isolated blastomere gives rise only to those parts which develop from it in intact embryos, that the cytoplasmic regions visibly distinguishable represent organ-forming substances, and that ascidian development is a mosaic. However, both Conklin and Chabry found that the sensory primordium might appear in right or left partial forms, and Conklin regarded this as a regulation. Development of one to three papillae in half-larvae, three being the number in wholes, was noted by G. A. Schmidt (1931) and regarded as indicating regulation. Development of isolated blastomeres was also studied by Berrill (1932), who agrees with Conklin; but later it was found that in half-larvae of *Ascidella* papillae vary in number from none to three (Cohen and Berrill, 1936). These authors admit the possibility of papillar reconstitution but regard it as more probable that a papillar primordium is variously divided or brought entirely into the right or left blastomere by slight variations in plane of first cleavage. They also consider it probable that presumptive neural tissue may become general ectoderm and take part in the covering of the half-larvae; and they find, as did Chabry and Conklin, that a sensory primordium may appear in right or left half-larvae, but offer various suggestions to account for this in terms of mosaic development rather than reconstitution.

Pieces of the unfertilized egg of *Ascidella* can be fertilized and develop (Dalcq, 1932*a, b, c*). Pieces resulting from meridional section may develop half-embryos, complete symmetrical forms, or forms with more or less complementary defects in the two halves. Dalcq, however, does not regard even development of a whole larva from a half-egg as "true regulation" but suggests that localization of cytoplasmic substances in the unfertilized egg gives it a bilaterally symmetrical structure and that meridional section in the median plane divides these substances symmetrically, so that all are present in each half and a whole larva develops from each. However, what would normally give rise to half of the symmetrical pattern gives rise in the half-eggs divided in the median plane to the whole symmetry pattern. To maintain that reconstitution does not take place in such cases seems scarcely in accord with the facts. Dalcq also finds that small portions may be removed from apical or basal polar regions without preventing normal development and that gastrulation occurs in pieces down to one-fifteenth the egg volume; but in apical pieces there is excess of ectoderm, in basal pieces ectodermal deficiency.

These results indicate a considerable capacity for reconstitution in the unfertilized ascidian egg. More recently Dalcq (1935, 1938*b*) has concluded that it is not possible to establish for ascidians a topographic locali-

zation of hypothetical cytoplasmic substances agreeing with the data of observation, that the occurrence of regulation in the ascidian egg must be admitted, and that ascidian development, like that of other forms, must be interpreted in dynamic terms. He regards the organization of the ascidian egg as consisting of a polar gradient, the mesoplasm (Conklin's yellow crescent), and a cortical, dorsiventral field with greatest concentration in the region of the equator. These are regarded as dynamic factors and morphogenesis is conceived as resulting from their interaction.

Eggs from which extrusion of cytoplasm has been brought about by puncture of the chorion and pressure may give rise to more or less normal larvae (Reverberi, 1931), but what region of the cytoplasm is removed in these experiments is not known. Isolation of the four apical and the four basal blastomeres of the eight-cell stage results in alteration of later cleavage in the former but not in the latter, and the conclusion is drawn that the factors controlling bilaterality are localized in the basal region (Reverberi, 1933). Pieces of the unfertilized egg of *Ciona*, when fertilized, cleave like the whole egg, irrespective of the region which they represent (Reverberi, 1936). Pieces of fertilized eggs with known orientation of plane of section give different results. Pieces above a certain size, obtained by section parallel to the polar axis, cleave like whole eggs. Pieces obtained by section vertical to the polar axis cleave like wholes if they contain a zone localized in three-fourths of the basal hemisphere; but pieces of the apical hemisphere, or of this plus the adjoining basal fourth, cleave radially. These experiments also indicate that factors determining bilaterality are localized in the basal region (Reverberi, 1937).

These isolation experiments show that developmental potencies of at least some regions of ascidian eggs are less strictly limited than most earlier investigators believed. Experiments on translocation and combination of blastomeres of *Ascidella aspersa* provide further evidence that this is the case (Tung, 1934). These experiments include rotations of the four apical blastomeres (micromeres) of the eight-cell stage 90° , 160° , and 180° on the four basal blastomeres, superposition of two groups of micromeres with polar axes unchanged but with various degrees of rotation about this axis, superposition of two two-cell stages with median planes at right angles, and various isolation experiments. The only parts which appear truly stable in Tung's experiments are the primordia of notochord, myoblasts, and probably of mesenchyme. Presumptive ectoderm and entoderm are "relatively equipotential." The four apical micromeres, normally ectodermal, can develop as entoderm; and the basal macromeres,

which normally produce little ectoderm, give rise to forms completely covered with ectoderm. Level of gastrulation is not predetermined; and Tung suggests a double polar gradient, animal and vegetal, such as Runnström has postulated for the sea urchin, and a quantitative determination of ectoderm and entoderm in relation to gradient-level. Here, as in the sea urchin, the vegetal gradient may be secondary, at least as far as dynamic factors are concerned.

According to Tung, the micromeres give rise to cerebral vesicle, and macromeres to neural cord in many cases; and presence of notochord is not necessary for formation of a cerebral vesicle, but the possibility of induction by entoblast is not excluded. Presumptive neural tissue may develop as general ectoderm, and supernumerary cerebral vesicles may develop. Presence, absence, and variable numbers of pigmented cells indicate that they, too, are not fixedly predetermined. The adhesive papillae also are apparently determined in relation to other parts.

Results of fusions, killing, and isolation of blastomeres and blastomere groups have led von Ubisch to conclude that the general organ-forming regions of the embryo, except ectodermal, are determined at the sixteen-cell stage. Presumptive neural ectoderm may become epithelium, and presumptive epithelium may become entoderm. However, the embryonic regions are not sharply separated from each other but are gradients which merge into each other (*ineinander übergehen*) at their boundaries; these boundary regions may develop into organs of either of the gradient systems concerned. Within the general organ-forming regions determination is not fixed at the sixteen-cell stage, but extensive reconstitutions (*Regulationen*) are possible.²¹

In short, it seems to be evident that ascidian development, often regarded as the most extreme case of mosaic development, is actually far from a mosaic in early stages. As in various other eggs and embryos, certain parts are more stable than others and appear thus far to be definitely restricted in potency; but the possibility remains that with other experimental alterations of environmental relations of these parts other potencies may be brought to light. Moreover, it appears that neither determinate cleavage nor partial development of isolated parts provides an adequate basis for the conclusion that ascidian development is, from the beginning, a mosaic of independent parts. As development progresses,

²¹ Von Ubisch, 1938b, 1940, also 1939, "Über die Entwicklung Ascidienlarven nach frühzeitiger Entfernung der einzelnen organbildenden Keimbezirke," *Arch. Entw'mech.*, 139; and a later paper, 1940, "Regulation und Determination im Ascidienkeim," *ibid.*, 140. See also the evidence for presence of an inductor region in ascidians on p. 480.

the mosaic condition is more nearly approached. Removal of various parts of the embryo preceding gastrulation affords no certain evidence of reconstitution, except possibly of entoderm, or of induction (von Ubisch, 1940).

CLEAVAGE AND PATTERN IN *Amphioxus*

Development of *Amphioxus* shows similarities in general regional pattern to ascidian and amphibian development. The apical hemisphere is chiefly ectodermal, the basal region entodermal; a mesodermal crescent lies posteroventrally, and opposite this is the area from which notochord and neural plate develop.²² In the separation of mesodermal and chordal areas this pattern resembles the ascidian and differs from the amphibian pattern. Evidence of dorsiventrality appears in the undivided egg, and the first cleavage plane usually coincides with the median plane; but cleavage is, in general, less distinctly determinate than in ascidians and, except for differences resulting from the smaller amount of yolk, gastrulation resembles amphibian gastrulation.

Development of isolated blastomeres led E. B. Wilson (1893) to the conclusion that *Amphioxus* development is not mosaic. Conklin (1933) agrees with Wilson that whole normal larvae may develop from 1/2 blastomeres but maintains that this is possible only if the blastomeres are right and left halves. Partly separated blastomeres of the two-cell stage show various degrees of twinning, the two partially united forms apparently developing independently of each other, often with polar axes in different directions in consequence of blastomere rotation. Partial separations at the four-cell stage develop into three or four blastulae or gastrulae, but further development is rare and abnormal. Conklin regards *Amphioxus* development as essentially mosaic, except for reconstitution of 1/2 blastomeres into whole individuals. However, in the light of the ascidian experiments, it seems possible that further experiment may lead to somewhat different conclusions: an inductor region may be concerned in *Amphioxus* development, and transplantations and combinations of blastomeres may bring to light relations of parts not shown by isolations.

CLEAVAGE AND DEVELOPMENTAL PATTERN IN OTHER FORMS

In many animals cleavage pattern is less definitely determinate than the patterns discussed or apparently entirely or almost entirely indeterminate. In forms with total cleavage (holoblastic) the planes of early divi-

²² Conklin, 1932. References to earlier literature given there.

sions are usually definitely related to the polar axis, those of the first two cleavages usually passing through this axis but often without definite relation to a median plane. In most animals with meroblastic cleavage little or no constant relation between cleavage pattern and pattern of development has been discovered. Evidently developmental pattern may be entirely independent of particular cells. Even in sea-urchin development, although ectoderm, entoderm, and mesenchyme develop normally from particular cells or regions, any one of the three parts can develop as either of the others in the earlier stages. Normally the cleavage appears to be, at least to a considerable degree, determinate; but nothing is definitively determined in early stages. Ectodermization of prospective entoderm, entodermization of prospective ectoderm, mesenchyme formation from prospective ectoderm or entoderm, and development of prospective mesenchyme as ectoderm or entoderm, all occur under experimental conditions. Dorsiventrality and polarity may be altered and even obliterated, although the cleavage pattern of earlier stages may remain entirely unchanged. Evidently there is no necessary relation between cleavage pattern and developmental pattern here. In reconstitutions in adult multicellular forms the cell is evidently not an essential factor in developmental pattern; the pattern is supercellular. It is becoming increasingly evident that, even in the so-called "mosaic forms," the relation of cleavage pattern and the cell to developmental pattern is often much less definite than has been assumed. In fact, even these forms are evidence of "the inadequacy of the cell theory of development" (Whitman, *Woods Hole Biol. Lect.*, 1893). Most botanists are agreed that developmental pattern in plants is not primarily a mosaic of cellular units of organization and function. Years ago De Bary said: "Die Pflanze bildet Zellen, nicht die Zelle bildet Pflanzen." The progress of botanical research has led many others to essentially similar conclusions. A recent expression of the view that plant organization is determined by the organ or organism, not by the cell, is the summary of studies in size and form of ovaries and fruits of Cucurbitaceae in the symposium paper by E. W. Sinnott (1939, "The cell-organ relationship in plant organization," *Growth*, Suppl.).

EFFECTS OF CENTRIFUGAL FORCE ON CLEAVAGE AND DEVELOPMENTAL PATTERN

Visible granules regionally localized in eggs and regional differences in appearance of cytoplasm have often been regarded as representing formative substances, but centrifuge experiments have shown that in most

cases developmental pattern is independent of the stratified distribution of these substances by centrifugal force. Stratification into three, four, or five more or less distinct layers or zones of different specific gravity and often of very different appearance results from centrifuging, according to the material and intensity of force. On cessation of centrifuging a gradual reconstitution of normal cytoplasmic structure usually tends to occur, except after very high intensities. In consequence of displacement of nucleus or spindle, one or both polar bodies may form elsewhere than normally, and cleavage pattern may be altered.

In the *Crepidula* egg, centrifuged at first polar spindle stages, the spindle may be displaced as a whole or greatly elongated; and when division occurs, the cell which would normally be the polar body may be as large as, or even larger than, the other cell. One or both polar bodies may be enlarged in this way, but even with abnormal size and position of polar bodies cleavage gives evidence of persistence of pattern, presumably the original pattern (Conklin, 1917).

In some eggs containing much yolk, oriented with polar axis in the direction of the force, apicobasal differences in rate of cleavage may be increased or reversed with aggregation of yolk at one pole or the other. Amphibian eggs centrifuged with heavier vegetal pole centrifugal may approach or attain meroblastic cleavage because the dense aggregation of yolk prevents cleavage in the centrifugal region. When centrifuged in reverse orientation, cleavage may become more rapid in the basal than in the apical region.²³ Such effects are primarily mechanical, and the aggregation of yolk may make normal development mechanically impossible; but this gives no information concerning physiological pattern. However, even with marked stratification in the amphibian egg, the blastopore is normally localized, and development may be essentially normal (Morgan, 1906c). Apparently the region of the dorsal lip has not been displaced.

Many eggs do not become axially oriented to centrifugal force, or orientation can be prevented; consequently, stratification may be at any angle to the axiate pattern, but a normal developmental pattern may appear even with a distribution of cytoplasmic components entirely different from the normal and different in different individuals.

Developmental pattern in the few coelenterate eggs centrifuged is apparently quite independent of degrees of stratification or alterations of cleavage resulting (Conklin, 1908; Beckwith, 1914). Complete or almost complete independence of developmental pattern and stratification is par-

²³ O. Hertwig, 1898b, 1904; Wetzel, 1904.

ticularly clear in eggs of certain annelids and mollusks, in which both a normal cleavage pattern and normal larval differentiation may be quite independent of the axis of stratification.²⁴ Eggs of the gasteropod *Ilyanassa*, centrifuged in reverse orientation, form the temporary polar lobe, although, except presumably for the cortex, the cytoplasmic contents of the lobe are quite different from its normal contents. In eggs separated into two parts by centrifuging, the lobe forms in the basal part, and rhythmic changes occur, even though no nucleus is present. Lobe formation and activity are apparently associated with conditions in the cortex, which is not displaced in centrifuging (Morgan, 1935, 1936). With sufficient centrifuging, eggs of the gasteropod *Physa* become greatly elongated and separate into pieces corresponding more or less closely to the stratification. Nucleated pieces, consisting only of clear protoplasm, may develop to young normal snails and hatch (Clement, 1938).

Effects of centrifuging on developmental pattern are apparently greater in eggs of the oligochete *Tubifex* and the leech *Clepsine*. The polar plasms (p. 550) become partly or wholly mixed with other cytoplasmic components, and development is not normal.²⁵ These eggs orient in the centrifuge with apical pole centrifugal; but, according to Schleip, the ectoplasm or cortical layer with the denser parts of the polar plasms attached to it becomes secondarily oriented independently of the entoplasmic stratification, so that a line joining the two polar plasms forms a right angle with the axis of stratification.

High-speed centrifuging induces or influences ventrodorsality in the egg of the geophyean *Urechis*, a form with spiral cleavage, the centripetal region tending to be ventral (Pease, 1938). In ultracentrifuged eggs of *Cumingia* and *Chaetopterus* cleavage pattern is related to stratification, though with wide variation in *Cumingia*, and polarity and ventrodorsality are apparently determined in relation to cleavage pattern (Pease, 1940, "The influence of centrifugal force, etc.," *Jour. Exp. Zool.*, 84).

With moderate centrifuging preceding cleavage, the *Ascaris* egg does not orient, the cleavage pattern is entirely independent of the stratification, and the amount and kind of the stratified substances in particular cells differ in different individuals and are without effect on development. Even after separation of "yolk balls" of considerable size, cleavage and

²⁴ See, e.g., F. R. Lillie, 1906, 1909; Conklin, 1910; Morgan, 1910b.

²⁵ *Tubifex*: Penners, 1922, 1924b, 1925; von Parseval, 1922. *Clepsine*: Schleip, 1914a, b, 1929, pp. 128-31.

developmental pattern in the remainder of the egg may show no fundamental alteration (Hogue, 1910). With stronger centrifuging continued from the stage of separate pronuclei through the first cleavage, the eggs are flattened by the force, the first cleavage spindle lies at right angles to direction of the force, and the first cleavage plane coincides with direction of force. In many of these eggs a cytoplasmic ball containing the heavier granules separates at the centrifugal pole during the first cleavage (Fig. 183) because the cleavage furrow does not penetrate this dense region (Boveri, 1910a, b; Hogue, 1910). Both cells resulting from first cleavage of these eggs behave like the ventral cell, P_1 (Fig. 179), as regards absence of chromatin diminution and later cleavage pattern. According to Boveri

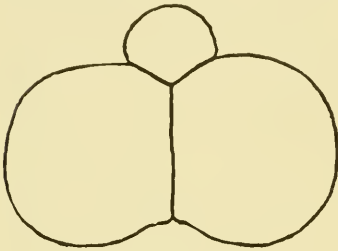


FIG. 183.—Separation of a cytoplasmic ball at first cleavage of strongly centrifuged *Ascaris* eggs (after Boveri, 1910a).

(1910b), occurrence or nonoccurrence of chromatin diminution depends on level of the cytoplasmic gradient at which nuclei lie. In the "ball eggs" both nuclei resulting from first cleavage are at the same level; consequently, they behave in the same way, and the level is evidently sufficiently basal so that diminution does not take place. Since the two blastomeres of these eggs include the whole, or almost the whole, polar gradient, their cytoplasmically determined nuclear condition may

play a part in determining their behavior as ventral cells. Ultracentrifuging *Ascaris* eggs usually suppresses the first cleavage, but nuclear division continues, and all nuclei usually undergo diminution (King and Beams, 1938). To account for these results a specific chemical diminisher substance is postulated, formed from cytoplasmic substance, more concentrated apically, and produced slowly, so that normally the first cleavage occurs before it diffuses throughout the egg. With suppression of first cleavage it diffuses more or less throughout; consequently, with few exceptions, all nuclei undergo diminution. At present the hypothesis that diminution may be a reaction to nonspecific differences in metabolism seems equally plausible.

Eggs of *Cyclops* apparently do not orient in the centrifuge but become stratified and elongate in direction of the force. The first cleavage spindle is parallel to the stratification; consequently, the first cleavage is in the greatest diameter of the egg rather than in the smallest, as normally, but

development is normal (Spooner, 1911). Does cleavage pattern determine developmental pattern in these cases, as it seems to normally?

Ascidian developmental pattern may be much altered by centrifuging, either by displacement of cytoplasmic components and resulting altered localization of particular differentiations or by abnormal cleavage pattern or by both. Neural tissue, sensory pigment cells, notochord cells, muscle cells, and cells resembling entoderm may be found, after centrifuging, in various regions quite different from the normal (Duesberg, 1926; Conklin, 1931).

Regional differences in specific gravity in the egg of the sea urchin *Arbacia* are not sufficient to determine orientation in the centrifuge. When the egg is centrifuged after fertilization, the first cleavage plane is vertical, the second parallel, the third again vertical to the plane of stratification; and the micromeres, normally at the basal pole, form about the intersection of two of the three cleavage planes, but their position may be centrifugal or centripetal or lateral in relation to the stratification, and the stratified constituents are differently distributed in different larvae, development being normal. Polarity of egg and embryo is evidently independent of the stratification.²⁶

With strong centrifugal force and a medium of proper density (sugar solution), unfertilized eggs of *Arbacia* undergo stratification, elongate in direction of the force, become dumbbell shape, and then separate into a colorless and a pigmented portion. In spherical stratified eggs, the first cleavage plane is usually perpendicular, in elongated eggs, usually parallel to the stratification. The dumbbell-shaped eggs, when fertilized, give normal plutei. Both colorless and pigmented portions can be fertilized and cleave and may develop to plutei. Both portions can be again separated by further centrifuging into two parts; these cleave on fertilization, but development does not proceed far. Activated anucleate halves or quarters can cleave and form blastulae (E. B. Harvey, 1932, 1936, 1939).

That the primary developmental pattern of the insect egg is localized in the superficial cytoplasm, or in a certain region of it, seems evident from the superficial character of the blastoderm. Stratification of substances by centrifuging in eggs of certain Coleoptera (Hegner, 1909) and muscid Diptera (Pauli, 1927) before blastoderm formation may be fol-

²⁶ Lyon, 1906, 1907; Morgan and Lyon, 1907; Morgan and Spooner, 1909. Morgan and Spooner believe that the original polarity may be somewhat altered by the effect of stratification on cleavage but that position of micromeres and larval polarity is as nearly coincident with the original axis as altered cleavage permits.

lowed by normal or almost normal development. With extreme degrees of stratification in the longitudinal axis of the beetle eggs, a dwarf larva develops from the plasmatic part, the centrifugal yolk mass remaining undeveloped or including some embryonic tissue without definite pattern. At the blastoderm stage and later, centrifuging has little effect in altering distribution of substances. Normal, or almost normal, embryos may also develop from centrifuged muscid eggs, but with sufficient centrifuging the embryonic zone is intermediate between a still lighter centripetal and a heavier centrifugal zone; these may remain without development or be taken into the embryo.

The cortical cytoplasm in which the blastoderm forms after nuclei migrate into it from the interior of the egg may be displaced toward one or the other end of the egg, but the fact that the embryos developing from centrifuged eggs show normal axial orientation indicates that the original pattern persists. Moreover, with centrifuging beginning in stages when few nuclei are present, these may be displaced centripetally so that only the centripetal part of the plasmatic zone becomes nucleated, and blastoderm and embryo are limited to this region. This is apparently development of a whole pattern from a part of the original pattern, that is, a reconstitution, and suggests, as do the other reconstitution experiments with insect eggs, that pattern at this stage may be little or nothing more than a quantitative differential.

It is sufficiently evident from the data mentioned that developmental pattern in many eggs is, to a high degree, independent of stratification of visible cytoplasmic constituents or inclosures. Alterations of cleavage by centrifugal force may be due to displacements of nucleus of spindle or to mechanical obstacles to cleavage resulting from aggregation of yolk. Obviously, the stratified substances are, in most cases, not "formative substances," and formative pattern is independent of their distribution. This pattern, then, must be a property of the "ground substance" (F. R. Lillie, 1909), supposedly not displaced by centrifuging; or it must be localized in the cortex or superficial cytoplasm of the egg, in which displacement or stratification of substances apparently does not result from centrifuging. Differential susceptibility and differential dye reduction seem to indicate that, in at least some eggs, a polar gradient pattern is present throughout the egg before the cytoplasmic movements associated with maturation and fertilization begin. If this is the case, this pattern perhaps persists only in the superficial cytoplasm, which is less, or not at all, involved in the streaming of deeper layers or in the stratification by centrifugal force.

Gradient pattern and morphological pattern in ciliate protozoa are limited to the ectoplasm, and results of centrifuging eggs indicate that developmental pattern may be continuously present only in the cortex, though perhaps extending to deeper cytoplasm in the oöcyte. If centrifuging involves the cortex or cytoplasmic areas closely associated with it, such as the polar plasms of *Tubifex* and *Clepsine*, developmental pattern may be altered. It appears, however, that ultracentrifuging may alter ventrodorsality and even polarity in some eggs with spiral cleavage, but whether the alteration results from effect on the cortex or on the deeper cytoplasm remains to be determined.

If a physiological gradient pattern involving metabolism is continuously present in the cortical cytoplasm, the hypothesis of an elastic or contractile network, which is merely stretched or distorted by displacement of yolk and other substances in centrifuging and brings the particles back into place after the force ceases to act, seems scarcely necessary. Assuming presence of a gradient pattern, the original distribution of cytoplasmic granules or inclusions and its changes in maturation, fertilization, and cleavage are undoubtedly related in some way to this pattern. It appears probable that differences in condition at different gradient-levels, perhaps extending to the deeper cytoplasm, may bring about the return to normal structure and distribution. Electric-potential differences and differences in surface tension and in colloidal dispersion are possible factors.

ALTERATION OF CLEAVAGE BY OTHER FACTORS IN RELATION TO DEVELOPMENT

It is a well-known fact that mitotic spindles tend to orient vertical to the direction of mechanical pressure and in the greatest cell diameter, though other factors may prevent such orientation in many cases. The stratification resulting from centrifuging is apparently very similar to mechanical pressure in its effect on orientation of spindles in many cells. Alterations of cleavage pattern and dislocation of blastomeres have been produced in various eggs by pressure and in some by other means, such as violent pipetting, shaking, and exposure to calcium-free sea water.

Cells of the early blastula of a medusa, *Aegineta*, after extreme dislocation by pipetting, tend to reconstitute a spherical blastula (Fig. 184) and to give rise to normal individuals (Maas, 1901). The apparent polar difference in size of blastomeres in Figure 184, *B* and *C*, suggests that a more or less normal arrangement may be regained. If a gradient pattern is present in the blastula, as in other coelenterate blastulae (pp. 96, 167),

potential differences or differences in surface tension in cells of different gradient-levels may bring the cells back to something like the original order. Moreover, polarity in embryonic development of various coelenterates is highly labile, and it is possible in this case that cells of higher gradient-levels may be sufficiently dominant to redetermine gradient-level in other cells "out of place." In certain other medusae (*Geryonia*, *Liriope*) calcium-free sea water and violent pipetting are necessary to

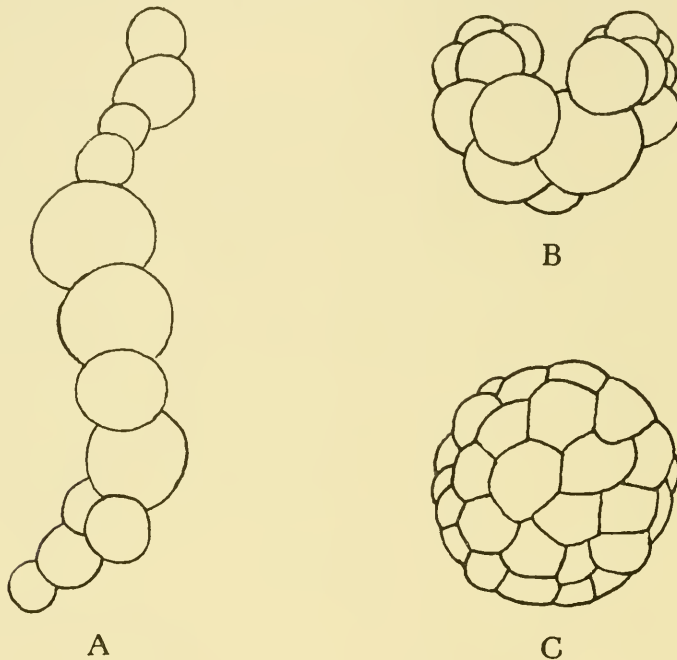


FIG. 184, A-C.—Dislocation of blastomeres of medusa, *Aegineta*, and reconstitution of spherical blastula (after Maas, 1901).

bring about dislocation of blastomeres, and development is more or less abnormal. The difficulty of dislocation is apparently due to physical properties of the blastomere surfaces, and these may also prevent rearrangement, or blastomeres may be injured by the experimental procedure.

When the sea-urchin egg is subjected to a certain degree of pressure, cleavage planes are in the direction of pressure, and eight- and sixteen-cell stages are flat plates of blastomeres only one cell thick; micromeres may not appear at the sixteen-cell stage. If the membrane is present, these plates become more or less rounded after pressure is removed, apparently

because the membrane tends to return to its original spherical form. In absence of the membrane, removal of pressure results in divisions vertical to the previous direction, so that the plate becomes two cells thick. Eggs with cleavage pattern thus altered may develop into normal plutei.²⁷ From these modifications of cleavage pattern Driesch concluded that blastomeres of the sea-urchin embryo are equivalent and can be mixed in any order without preventing normal development. A critique by Braem (1893) showed this conclusion to be unnecessary, and these experiments are now generally regarded merely as evidence for independence of early cleavage pattern and developmental pattern. If developmental pattern is a general or a cortical gradient pattern, these changes in cleavage pattern may not affect it at all, and even a regionally specific pattern might persist essentially unaltered by the changes in cleavage.

Even in nemerteans, annelids, and mollusks—forms with spiral cleavage—pattern of early cleavage can, to some extent, be dissociated from developmental pattern. In eggs of species of all three phyla under pressure, cleavage spindles tend to lie in the greatest diameters of the eggs, that is, vertical to direction of pressure, and plates of eight or sixteen cells may result, but the egg polarity is also a factor in determining cleavage pattern in these experiments. For example, in eggs of the nemertean *Cerebratulus*, with pressure at right angles to the polar axis, the first cleavage plane passes through polar and pressure axis; the second, at right angles to the first, also passes through the pressure axis, that is, it is equatorial with respect to the polar axis, instead of meridional like the second normal cleavage. If pressure is removed at this stage, the third cleavage planes are meridional, like the second of normal development, and further development is normal. Plates of eight or sixteen cells may also result from pressure, but pilidia developing from these are more or less defective or abnormal, sometimes partial duplications involving the apical organ (Yatsu, 1910a, b). According to Dederer (1910), polarity of the *Cerebratulus* egg can be altered by pressure; but since no other experimenter has observed such alteration in forms with spiral cleavage, this may be an error.

Polar-body position is not altered in the egg of the mollusk *Cumingia* by pressure in any direction, and the first cleavage plane usually passes through the polar axis, though with pressure at right angles to this axis, it is sometimes oblique or equatorial (Browne, 1910). With sufficient pressure in the direction of the egg axis of some other forms with spiral cleav-

²⁷ Driesch, 1892; Morgan, 1894; Ziegler, 1894.

age, the third cleavage is meridional, instead of equatorial, and a plate of eight cells is formed. On removal of pressure at this stage, the eight cells, or most of them, particularly those including the region about the apical pole, give rise to micromeres by divisions vertical to those that formed the plate.²⁸ Normal trochophores may develop from these forms (Wilson); but, according to Morgan, all are at least slightly abnormal.

Alteration of cleavage pattern by pressure in the ascidian *Ciona* practically always results in abnormal development (Morgan, 1910a). Here cleavage pattern is apparently more closely associated with developmental pattern. Only the mutual relations of the first three cleavage planes of the ctenophore egg have thus far been altered by pressure. Unequal divisions occur under pressure, as normally, but later development has not been followed (Ziegler, 1898). In the egg of *Ascaris* little change in cleavage pattern has resulted from pressure (Girgolauff, 1911; Bonfig, 1925).

Since it was discovered by Herbst (1900) that in calcium-free sea water blastomeres of sea-urchin eggs do not flatten against each other but remain spherical and may become completely separated, this procedure has been widely used as a means of obtaining isolated blastomeres or blastomere groups and also in some cases for dislocation of blastomeres. Calcium-free sea water alters condition of the ectoplasmic layer which holds the blastomeres together. On return to normal sea water the change is more or less completely reversed, and blastomeres again adhere normally. Herbst observed that cell division continued in the calcium-free water and that even isolated cells might become ciliated. Somewhat later Driesch (1902a) found that dislocation of blastomeres, varying from one individual to another, could be obtained by exposure to calcium-free water and that, on return to normal, sea water development proceeded. When not disturbed, sea-urchin eggs in calcium-free water tend to form a cell plate; and apical or basal cells, normally in contact, may be widely separated. Dislocation of the four basal cells of the eight-cell stage into two separated pairs results in formation of two groups of micromeres; and, if these remain separated after return to normal sea water, two invaginations and two archentera may develop. These results are in line with those of Hörstadius on transplantation of micromeres (pp. 440-45). In spite of extensive dislocations of blastomeres, however, many of the more or less platelike blastomere groups develop into normal plutei. There is considerable change in cell positions as the mass becomes rounded on return to

²⁸ *Nereis*: E. B. Wilson, 1896; Morgan, 1910a. *Crepidula*: Conklin, 1912.

normal sea water, and how far return of cells to normal relations takes place is uncertain. Driesch maintains, however, that normal development may result, although blastomeres, except the micromeres, remain variously dislocated, and agrees essentially with Boveri (1901*a, b*) that the vegetal region determines other parts. Since entodermization of prospective ectoderm, ectodermization of prospective entoderm and micromeres, and induction of invagination from prospective ectoderm by implanted micromeres have been shown to take place, normal development should be possible with considerable dislocation of blastomeres.

Cerebratulus eggs from which membranes have been removed by shaking give rise in calcium-free sea water to a ring of eight cells, or a plate of two rows of four each, or in some cases to eight cells in a single series (Yatsu, 1910*b*). In all these the third cleavage, like first and second, is vertical to the underlying surface. On return to normal sea water at this stage, all eight cells may give rise to micromeres and rings; plates and single rows of cells may develop into pilidia, some of which appear normal, while in others the apical organ is absent or doubled. Either the dislocated blastomeres regain a more or less normal arrangement, as Yatsu suggests, or there is partial redetermination of pattern by one or more dominant regions. *Cerebratulus* embryos, kept in calcium-free water for longer periods, form solid irregular cell masses with little visible differentiation, except an apical flagellum and ciliated cells in some. Returned to normal sea water, these masses differentiate ectoderm and gut but do not attain full pilidium development. Some of Yatsu's figures suggest differential inhibition of development, greater in ectoderm than in entoderm.

ALTERATIONS OF CLEAVAGE BY DISPERMY AND POLYSPERMY

Effects of dispermy and polyspermy have been most extensively studied in sea-urchin eggs. The first cleavage of a dispermic egg may be with two separate spindles into two cells, or with monasters or various forms of multipolar spindles into three or four cells simultaneously, and the patterns of following cleavages differ from normal. Normal plutei develop from only a small proportion of these eggs, and often development does not continue beyond blastula or gastrula stages. Many of the forms resemble those resulting from differential inhibition by external agents. The blastocoel is often more or less filled with free cells, as in differentially inhibited forms; and cells may also be given off externally, also as in differential inhibition (chap. vi). Cytological studies by Boveri showed that

the chromosomes are usually abnormally and variously distributed in the first cleavage of dispermic eggs, so that some cells obtain less, some more, than the normal number.²⁹ Boveri pointed out that the abnormal development could not be due to cytoplasmic factors because $1/4$ blastomeres of normal eggs can give rise to normal larvae, and concluded that it constituted proof of qualitative difference of different chromosomes. However, according to Schleip (1929, p. 473), frequency of normal development of isolated primary three or four blastomeres of dispermic eggs is far below expectation on the basis of chromosome distribution. Boveri explained this low frequency as due to injury in separation of blastomeres; but Schleip suggests that, in addition to abnormal chromosome distribution and incidental injury, poor condition of eggs favors dispermy and may be in part responsible for abnormal development. But whatever the factors determining abnormal or differentially inhibited development in dispermic eggs, the occurrence of normal development in some whole eggs and isolated primary blastomeres shows that cytoplasmic pattern is not necessarily altered.

The modifications of cleavage pattern in dispermic eggs of the mollusk *Dentalium* have been described by Schleip (1925). With simultaneous cleavage into three or four cells, all may be in one plane or, in case of four cells, may form a tetrahedron with three apical and one basal, or one apical and three basal. Tetrahedral forms without definite relation to the polar axis were not observed. The swimming forms which develop from dispermic eggs of isolated blastomeres of these eggs are, according to Schleip, too abnormal to permit exact analysis.

Dispermy has been observed occasionally in *Ascaris* eggs and usually results in simultaneous division into four cells. Fusion of two or even three of these cells may occur, supposedly because chromosomes are absent between certain poles of the tetrapolar spindle. When the four cells persist, three types of second cleavage are distinguishable, as follows: three cells undergo diminution of chromatin, one does not; two cells undergo diminution, two do not; one undergoes diminution, three do not. The three patterns are indicated in Figure 185, *A-C*, the cells which undergo diminution being uppermost. In these patterns three, two, or one of the four cells correspond to *AB*, and one, two, or three, to *P₁* of normal cleavage, not

²⁹ Boveri, 1902, 1904, 1905, 1907. Morgan had suggested earlier that the high frequency of abnormal development in eggs dividing simultaneously into three or four cells, whether in consequence of dispermy or other conditions, resulted from difference in number of chromosomes in cells.

only as regards diminution but also as regards products of later cleavages (Boveri, 1910a, b). Boveri's conclusion that polarity in the *Ascaris* egg is a gradient was drawn from these cleavage patterns of dispermic eggs. Although the earlier normal cleavages of *Ascaris* are highly determinate, it appears from the dispermic eggs that a physiological cytoplasmic pattern persists independently of the alteration of cleavage pattern; but whether it is quantitative or regionally specific remains uncertain. Since

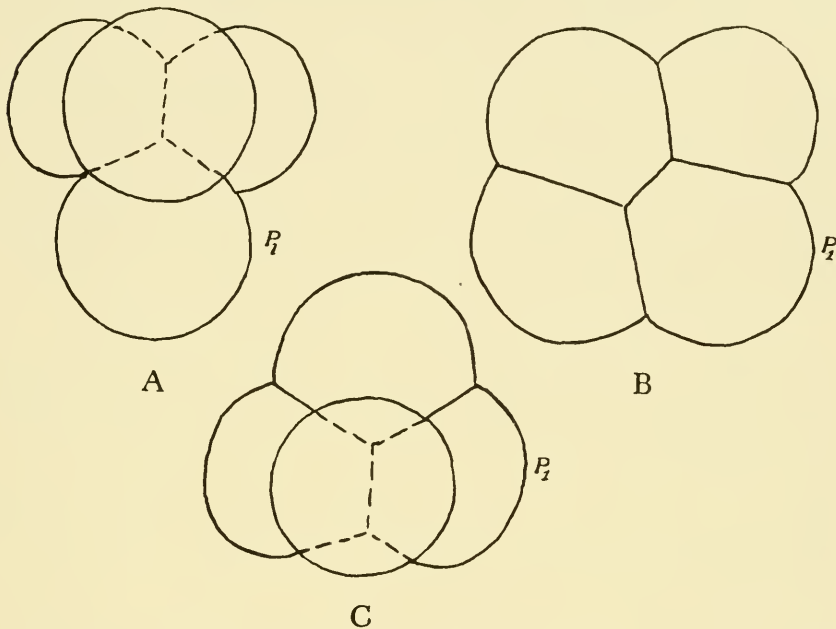


FIG. 185, A-C.—Diagrammatic outlines of cleavage of dispermic *Ascaris* eggs. A, three AB cells, one P_1 cell; B, two AB, two P_1 ; C, one AB, three P_1 (modified from Boveri, 1910b).

dispermic eggs give rise, not to normal individuals, but only to cell masses, it is evident that something is wrong with developmental pattern. According to Boveri, eggs with one ventral cell (P_1) develop more nearly normally than the other types.

Entrance of more than one spermatozoon occurs normally in eggs of urodele amphibians, but the supernumerary spermatozoa and their centers take no part in development and disappear. Experimental polyspermy in anuran eggs, resulting from high sperm concentration, alters cleavage because the number of centers equals the number of spermatozoa, but only one sperm nucleus unites with the egg nucleus. Di- and

trispermic eggs develop more nearly normally than others and may give rise to complete embryos and larvae, but there is often regional cessation of development and death of cells at some stage, and partial forms without reconstitution result. With extreme polyspermy, most of the embryo may die, or the egg may not cleave.³⁰ Death of cells, of regions, or of the whole embryo evidently results not from alteration of cleavage pattern but from the anomalous relations of nuclei and cytoplasm, cells being often multinucleate, and from abnormal and irregular distribution of chromosomes by multipolar spindles.

From the facts at hand it appears that dispermy, like centrifuging and pressure, may alter cleavage pattern without essentially altering cytoplasmic developmental pattern but that normal development rarely results, either because of pathological condition of eggs permitting dispermy or because of anomalies in chromosome distribution. Chromosome anomalies may alter or even obliterate developmental patterns by altering quantitatively or qualitatively the metabolism of cells or cell groups.

DIFFERENTIATION WITHOUT CLEAVAGE IN ANNELID EGGS

Unfertilized or fertilized eggs of the annelid *Chaetopterus* subjected for an hour to certain concentrations of KCl added to sea water may undergo some differentiation without cleavage or nuclear division (F. R. Lillie, 1902). The ectoplasm becomes vacuolated, as does the ectoderm of the trochophore, and in some cases a ring of large vacuoles girdling the egg resembles the ring of large vacuoles in the normal prototroch. Motile cilia develop on the whole or a part of the surface; and slow or active, but apparently undirected, swimming may occur. The yolk becomes aggregated into a dense mass in the interior, sometimes separated from the outer layer by a space. Cytoplasmic divisions may take place, but the nonnucleated masses apparently fuse again with the nucleated portion. Cilia develop somewhat later in these forms than normally.

Ciliation without cleavage and in one case a form with a girdle of cilia resembling the prototroch have also been obtained by KCl treatment of unfertilized eggs of the polychete *Podarke* (Treadwell, 1902). Unfertilized eggs of another polychete, *Amphitrite*, treated with $\text{Ca}(\text{NO}_3)_2$, KCl, or CaCl_2 or subjected to strong mechanical agitation, may also undergo differentiation without cleavage and without, or with, nuclear division, or with few irregular or partial cleavages and few nuclei (J. W. Scott, 1906).

In these three forms normal cleavage is typically spiral, but some dif-

³⁰ A. Brachet, 1910a, b, 1912; Herlant, 1911.

ferentiation is evidently possible, not only in absence of the normal cleavage pattern but in absence of any cleavage or nuclear division. Again it appears that, even in forms with spiral cleavage, cleavage pattern is not a fundamental factor in differentiation.

CONCLUSION

It is evident that early cleavage pattern and organismic developmental pattern may be completely independent or more or less closely associated. In some cases cleavage may perhaps be a factor in determining developmental pattern, but more commonly developmental pattern plays some part in determining cleavage pattern when the two are related. Even in some forms with determinate cleavage more or less alteration of cleavage does not necessarily alter developmental pattern. At present it appears highly improbable that any organism is, in any strict sense, a mosaic of self-differentiating cells or cell groups. At certain stages some parts may be more independent than others, but capacity for more or less independent differentiation when they are isolated is not proof that they are independent in the intact organism. Moreover, the postulated "organ-forming substances" do not exist. An organ is the product of an action system or various systems in relation to others. A multicellular organ involves orderly relations of cells and a definite spatial pattern. A particular substance may bring about differentiation of a cell or tissue; but how can it form an organ? Trochoblasts of the annelid embryo or the presumptive notochord of the ascidian may differ specifically from other parts, and their specific constitutions may determine their differentiation; but a prototroch or a notochord can develop only in relation to other parts. The formative factors in development are interrelated action systems.

Even in the most extreme cases early development approaches or attains mosaic character only as regards certain parts, largely those giving rise to temporary larval organs or apical or cephalic regions. Eggs of different species differ widely as regards degree or stability of regional determination at the beginning of embryonic development; but in no case known are all parts fixed and stable in their determination or differentiation, even in relation to isolation alone.

Once more it may be recalled that in many forms with relatively mosaic embryonic development this is only one of various ways in which individuals of the species may originate. For example, spiral determinate cleavage is characteristic of embryonic annelid development; but in many annelid species individuals can develop from buds, from fissions, or from

experimentally isolated pieces of the adult body, in some species even from a single segment. An ascidian can develop not only from an egg with highly determinate cleavage and apparent regional cytoplasmic differentiation but from buds of various origin, from isolated pieces of the adult body, from small pieces of stolons, and in many species from aggregations of cells, forming as other parts of the adult body undergo involution or degeneration. These forms of development may involve origin of new polarities, symmetries, or asymmetries, and localizations of primordia in definite and orderly relations to the new axiate pattern; but an organization and cleavage like that of the egg and early embryo are evidently not essential in these forms of development. Development from the egg is apparently the most highly specialized form of annelid and ascidian development. Egg organization at the beginning of embryonic development in such forms does not represent the real beginnings of individual development. Only by comparative investigation and analysis of the different forms of development can we hope to distinguish the fundamental factors in origin and development of individual pattern from those incidental to a particular form of development.

If annelid or ascidian embryonic development were a rigidly determined mosaic, budding, fission, and reconstitution of pieces would be impossible in later life. If dedifferentiation of cells is involved in these forms of development, the cells which dedifferentiate were not rigidly determined. If we assume that certain cells remain undifferentiated and are activated in some way in budding, fission, and reconstitution of pieces, those cells were certainly not rigidly determined.

In many organisms, even higher vertebrates, incapable, so far as we know, of giving rise in adult life to new individuals by budding, fission, or reconstitution of isolated parts, these forms of development are possible in early embryonic stages and have proved valuable aids in physiological analysis. It is, of course, far from true that every individual organism originates from an egg, and some of the other ways in which individuals originate may be more important than the egg and its development for attainment of an adequate concept of origin and nature of developmental pattern.

CHAPTER XV
QUESTIONS OF ORIGIN OF CERTAIN AGAMIC
PATTERNS UNDER NATURAL
CONDITIONS

CONCERNING various developmental patterns we know little or nothing beyond the fact of their appearance under natural conditions. They have been described, but experimental analysis is lacking. Some of them are practically inaccessible to present experimental methods; and only suggestions, hypotheses, or guesses as to their origin and nature are possible. These may serve, however, to make evident some of the problems they present and to indicate possibilities. Perhaps it is as important to call attention in this way to some of the things we do not know about development as to present established facts. Moreover, information concerning origin and nature of these patterns is no less, perhaps in some cases even more, essential to an adequate theory of development than information concerning embryonic development alone.

APPEARANCE OF AXIATE PATTERN FROM PLANT SPORES

Plant spores are usually unicellular but may be multinucleate without cellular division of the cytoplasm. Many kinds of spores appear, with different relations to the life-cycle: some apparently have nothing but surface-interior pattern, others show definite axiate patterns, and in some algae there seems to be little difference between spores and gametes.

The nonmotile spores of many fungi are spherical or ovoid bodies, often with no indications of axiate pattern. Germination usually consists in formation of a bud from one or more regions of the spore; this elongates, forming a mycelial filament, and gives rise to further new axes by budding, often without formation of separate cells. Germination apparently results from a local activation, as in other buds—perhaps in relation to some external differential or differentials. Presence of a gradient has been shown, at least in the apical regions of mycelial filaments of various fungi. In certain rusts (e.g., *Puccinia*) germination takes place through pores in the spore coat on opposite sides, so that axes arise in two opposite

directions. Here the pores through which the spore protoplasm pushes outward may serve to localize the outgrowth and so the axiate pattern.

Spores of bryophytes and pteridophytes differ in form, those of some species being spherical, others tetrahedral because of relation to sister cells, still others bilateral, and in some the spore coat (exospore) may rupture in a definite manner on germination; but whether a definite axiate pattern is determined in the spore protoplasm seems not to be known. Germination is apparently a budding, an activation, and an outgrowth of a region of the spore protoplasm, determined either by the region of rupture or by some local differential; or, if the spore possesses an axiate pattern, that presumably determines the region of initiation of outgrowth. The primary outgrowth may be a rhizoid, development of other axes occurring later. In the true mosses the moss plant originates as a bud from the filamentous protonema which results from spore germination. This type of development resembles the development of the hydranth and stem in many hydroids from stolonial outgrowths. Evidently the various buddings involved in these forms of development represent new polarities originating in local activations. Like buds of higher plants and of animals, they are probably primarily radial gradient systems and become axiate by differential growth of central and peripheral regions.

Spores of mosses and ferns develop from certain cells of sporangia, which are usually axiate. Whether spores ever possess a polarity derived from that of the sporangium seems not to be known; but if they do, it is probably readily obliterated and a new polarity determined by local external conditions. Certain of the pteridophytes are heterosporous, and the male gametophytes developing from microspores are reduced to a few cells inclosed by the spore wall but show a definite orderly pattern, suggesting a polarity. If a polarity is present, it is probably determined by the relation to each other and to free surfaces of the four spores of a tetrad. The female gametophyte of the heterosporous forms, *Selaginella* and *Isoetes*, on the other hand, shows a definite polar pattern in general coincident in direction with the polarity of the sporocarp axis on which it originates (e.g., F. M. Lyon, 1901).

In certain water ferns (Hydropterineae) an axiate sporocarp, arising essentially as a bud, consists of an outer covering (indusium) inclosing a branching axis with megasporangium apical and one-celled microsporangia at the tips of lateral branches. Development of any of the thirty-two cells in the terminal megasporangium inhibits development of the microsporangia; but if all cells of the megasporangium degenerate, the microspo-

rangia develop (Pfeiffer, 1907). This is essentially a multiaxiate sporangial pattern with apical region apparently dominant, as in multiaxiate vegetative stages of many plants.

All seed plants are heterosporous with reduced gametophyte generation, the pollen grain representing the male, and the cells of the embryo sac in the ovule, or, according to some botanists, the endosperm, representing the female gametophyte. Among the gymnosperms pollen grains may consist of several cells in a definite axiate pattern, and the question arises whether this is determined by the relation of the tetraspores to each other or by some other factor. Pollens of certain conifers possess two wings, developing on the outer surface of each tetraspore and symmetrical to the axis of the grain indicated by the cells composing it; that their localization and the axiate pattern of the grain are determined by the relation of each spore to the others in the tetrad appears probable.

In most angiosperms the two divisions of the spore mother cell form a linear series of four cells, coinciding in direction with the ovule axis, the innermost of these giving rise to the embryo sac. Polarity of the ovule apparently determines, in some way, the direction of the mitoses. The long axis of the embryo sac and, in the gymnosperms, of the archegonia developing in it also coincides with the ovule axis and apparently develops as part of it. The ovule itself is, in certain respects, similar to a bud in origin but has a symmetry pattern definitely related to the pattern of the ovary.

Motile swarm spores (zoospores) with flagella are characteristic of various algae and some fungi. The macrozoospore of *Ulothrix*, for example, is formed by division within a cell of the axiate filamentous thallus, which consists of a single cell series. One end of the zoospore is somewhat pointed and bears four flagella; an axiate differentiation is also present, consisting of colorless cytoplasm containing a contractile vacuole at the flagellate pole and chlorophyll-bearing cytoplasm elsewhere; on one side of the body is a red pigment spot, the so-called "eyespot," or stigma (Fig. 186, A). In short, as often noted, these zoospores resemble flagellate protozoa. The microzoospores (gametes) are similar in pattern, but they are smaller and have two flagella.

The same questions as to origin of this axiate, asymmetric pattern arise with reference to these zoospores as for the similar protozoan patterns. Certain early figures (e.g., Dodel, 1876) show the polarities of two macrospores formed within a thallus cell as parallel, oriented in the same direction at right angles to the thallus axis, and with pigment spots

adjoining, respectively, distal and proximal walls of the thallus cell (Fig. 186, *B*); but the spores in different mother cells of the same filament may differ in axial orientation. Single thallus cells may give rise to more than two spores, even to thirty-two or more microspores. In these cases the spores apparently form at the surface of the cytoplasm adjoining the cell wall, and their axiate patterns seem to be related to the differential from surface to interior of the protoplasm. Very similar spores with two flagella at one pole of an axiate pattern are formed in other green algae, and both or one of the gametes of some algae have similar axiate pattern. They

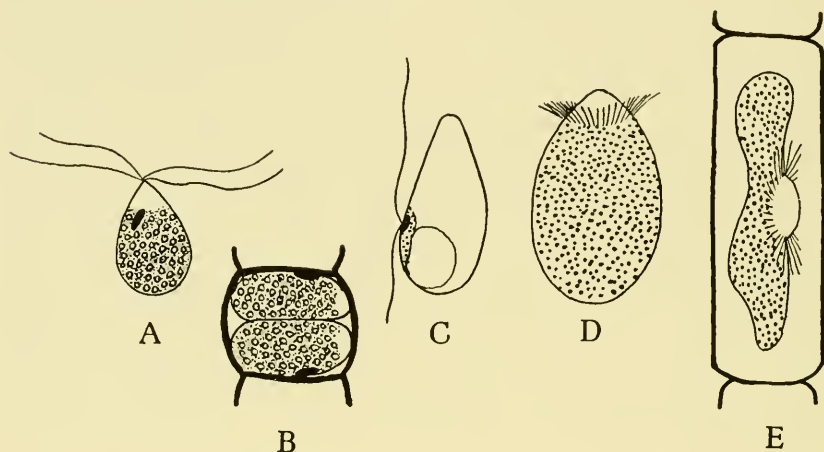


FIG. 186, *A-E*.—Zoospores of algae. *A*, zoospore of *Ulothrix*; cytoplasmic regions and pigmented "stigma" indicated; *B*, two zoospores developing in a thallus cell with axes transverse to thallus axis, parallel and similarly directed (*A* and *B*, diagrammatic, after Dodel, 1876); *C*, type of zoospore characteristic of brown algae; *D*, zoospore of *Oedogonium*; *E*, its development with axis transverse to thallus axis (*D* and *E* after Pringsheim, 1858).

usually develop by repeated divisions of thallus cells, often with formation of a very large number from a single cell. How the axiate pattern originates is an interesting question. In many forms the axes seem to be in all possible directions; certain figures, however, show the flagellate poles of spores lying superficially directed toward the surface of the mother cell,¹ but in most figures no such relation is evident. An axiate pattern related to, and determined by, directions of mitoses may perhaps become the polar axis of the spore, the polar region of the spindle becoming apical.

It is stated that zoospores of this type attach by the colorless flagellate

¹ See, e.g., G. M. Smith, 1938, Fig. 59, *Codium*.

pole, a rhizoid develops from it, and the chlorophyll-bearing portion becomes the thallus. Since the colorless flagellate pole is apical or anterior, as far as locomotion is concerned, this course of development suggests that the thallus filament represents a new polarity arising from the primarily basal pole, as the hydranth-stem axis of calyptoblast hydroids develops from the originally basal end of the planula (p. 96). These zoospores and gametes are usually asymmetrical as regards position of the stigma and apparently, at least in some forms, as regards nuclear position. Among the brown algae zoospores and gametes occur with two flagella arising from one side—the longer extending anteriorly, the shorter posteriorly, in relation to direction of locomotion—and stigma closely associated with point of flagellar origin (Fig. 186, *C*). Whether these asymmetries show any definite relation to particular factors in the environment of the developing spores is apparently not known.

In certain other algae—for example, *Oedogonium* and *Vaucheria*—a zoospore develops without division from an elongated cell of the thallus. The zoospore of *Oedogonium* possesses a circle of cilia near one end and a polar cytoplasmic differentiation into colorless and chlorophyll-bearing zone at the level of the cilia (Fig. 186, *D*). It appears, however, that this pattern develops at right angles to the thallus axis (Fig. 186, *E*), but the factor determining it is not evident. This spore also attaches by its colorless “apical” pole, from which rhizoids develop; and the thallus forms from the chlorophyll-bearing part.

The large multinucleate zoospore of *Vaucheria*, developing at the tip of a branch, becomes separated by a cell wall from the rest of the thallus, in which cell walls are absent, and becomes ciliated over the entire surface, two cilia developing above each of the numerous superficial nuclei. In germination, local budlike outgrowths, becoming new filaments, develop from one or more regions. Apparently at least some of these outgrowths represent new axiate patterns, resulting from local activations, determined by some internal or external differential.

In various algae the zygotes themselves or products of their division become oöspores, quiescent stages showing no indication of polarity and giving rise on germination to a bud axis in the same way as the agamic spores. Here, also, new axiate pattern apparently originates from a local activation. The fertilized egg of *Chara* becomes an oöspore; germination consists in the origin of a bud from some point of its surface; the bud becomes a filament; and the axis of the plant develops as a branch of this filament, that is, from another bud. It is sufficiently evident from these

few examples that patterns of various plant spores present interesting problems and also that development from spores often involves formation of new axiate patterns originating as buds. These, like other buds, are apparently primarily radial gradient systems resulting from local activations and become axiate by differential growth. Doubtless different gradient systems of this sort, even in the same plant, may differ chemically and physically and, consequently, may give rise to axiate patterns of different kinds. The case of the *Fucus* egg (pp. 423-25) suggests that many of these forms of reproduction and development still afford interesting fields for experiment.²

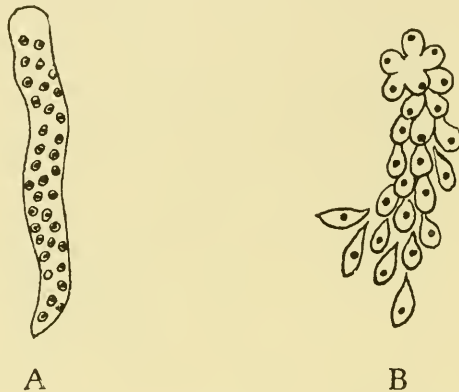


FIG. 187, A, B.—Schizogony of a schizogregarine, *Schizocystis gregarinoides*. A, multinucleate stage; B, formation of merozoites by cell-budding and separation about each nucleus, progressing from the posterior end anteriorly (after Léger, 1909).

PATTERN IN RELATION TO CELL FORMATION IN SPOROZOA

In many Sporozoa the formation of merozoites and sporozoites and, in some forms, of gametes shows an interesting relation between pattern and cell formation. The elongated uninucleate sporozoite of *Schizocystis gregarinoides*, a schizogregarine, develops into a wormlike form (schizont); this becomes multinucleate with nuclei coming to lie near the body surface (Fig. 187, A); a cell body buds from the parent body about each nucleus; separation into uninucleate cells results; and these elongate to form merozoites (Léger, 1909). The separation into cells occurs progressively from the posterior end anteriorly (Fig. 187, B), suggesting a slight

² References have been omitted from this section almost entirely except in connection with figures reproduced from papers, because the morphological data appear in botanical textbooks and because the purpose here is merely to call attention to a few examples of these patterns and the questions arising from them.

and decreasing dominance with physiological isolation progressing from the posterior end anteriorly. The merozoites may develop either into new schizonts or into gamonts which pair, encyst, and give rise to male and female gametes by nuclear multiplication and the budding-off of cells from a central mass. Two gametes unite, and nuclear multiplication and cell formation about each nucleus give rise to sporozoites which become schizonts (Fig. 187, *A*).

Another schizogregarine, *Caulleryella pipientis*, has the form of Figure 188, *A*, in the nonreproductive stage but becomes spherical before repro-

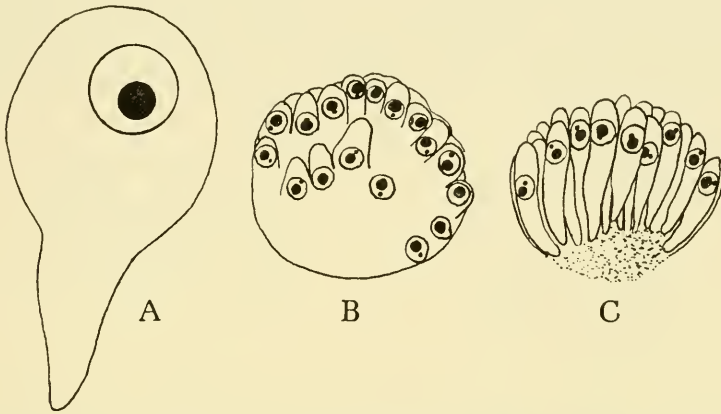


FIG. 188, *A-C*.—Schizogony of a schizogregarine, *Caulleryella pipientis*. *A*, uninucleate parasite; *B*, cell-budding about each nucleus; *C*, merozoites and cytoplasmic remainder (after Buschkiel, 1922).

ducing. Reproduction begins with nuclear multiplication; the nuclei approach the surface, mostly on one side of the spherical mass; and elongated cell bodies bud out from the region about each nucleus, becoming the merozoites and leaving behind a cytoplasmic remainder (Fig. 188, *A*, *B*, *C*). Gametes also develop from the gamonts as cell buds about nuclei, and sporozoite formation from zygotes is essentially similar to merozoite formation (Buschkiel, 1922). If there is any appreciable dominance in the uninucleate parasite, it is apparently lost before reproduction begins; but position of nuclei at the stage of cell-budding (Fig. 188, *B*) suggests a differential of some sort in the mass, perhaps persistence of traces of the axiate pattern of the uninucleate form (Fig. 188, *A*). Development of merozoites (Fig. 188, *B*, *C*) also suggests relation to a polar differential in the schizont from which they arise.

Gamete formation in the gregarines commonly takes place in a cyst containing two individuals. In each of these nuclear multiplication, sometimes to a great number, occurs, each individual in such cases becoming an irregular mass with extensive surface. The nuclei become superficial, and a gamete forms about each nucleus, one of the original individuals giving rise to microgametes, the other to macrogametes. Figure 189 shows a stage in formation of macrogametes (*A*) and microgametes (*B*) in a eugregarine. Superficial nuclear position apparently results from reaction to a surface-interior differential in the mass, and the outgrowth in relation to each nucleus is again a budding of cells from the common mass, apparently initiated either by the nucleus or by cytoplasmic factors closely associated with it, such as may be indicated morphologically by a centrosome or blepharoplast.



FIG. 189, *A, B*.—Stage in gamete development of a gregarine, *Urospora lagidis*. *A*, megagametes; *B*, microgametes (after Brasil, 1905).

Cell formation by budding from a multinucleate mass in relation to a nucleus is very generally characteristic not only of gregarines but also of Coccidia and Haemosporidia, and not only in development of merozoites and sporozoites but in that of gametes. A stage in development of microgametes of a coccidian is outlined in Figure 190, *A*. The fully developed microgamete is a greatly elongated, filamentous, biflagellate cell with undulating membrane and nucleus also elongated to filamentous form. The polarity arising in the bud which gives rise to this filamentous cell apparently determines its axiate pattern. Developmental stages of the microgamete of the tertian-fever parasite, a haemosporidian, indicated in Figure 190, *B*, show a similar cell-budding in relation to nuclear position; microgametes of other Haemosporidia develop similarly. In general, reaction to a surface-interior differential and a local budding of cells from a multinucleate mass are apparently concerned in origin of such axiate pattern as these forms possess.

The peculiar multinucleate spores of the subclass Cnidosporidia of the Sporozoa exhibit great variety of pattern in different species (Doflein-Reichenow, 1929). They possess a distinct polar organization, some are flattened in a plane of the polar axis, some are elongated at right angles to the polar axis, others have long, tail-like extensions of the envelope, and still others (order Actinomyxidia) develop a triradiate pattern about the polar axis, the envelope in some species giving rise to long slender processes and the whole spore resembling a three-armed or, in another species, a six-armed grappling hook. These spores possess one, two, three, or four polar capsules, each containing a spirally coiled thread which is extruded under certain conditions—for example, in some forms on exposure to intestinal fluids, or experimentally to various agents—and is supposed to aid in anchoring the spore. These capsules resemble nema-

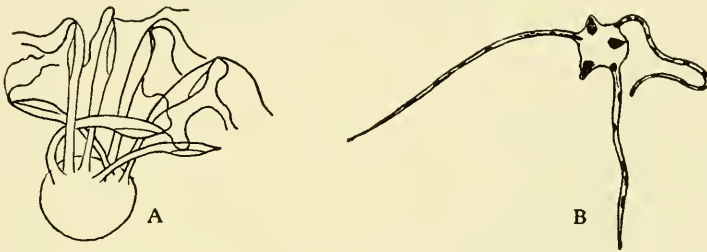


FIG. 190, A, B.—Development of microgametes of other Sporozoa. A, developmental stage of a coccidian microgamete, *Aggregata spinosa* (after Moroff, 1908); B, developmental stages of a haemosporidian microgamete, tertian fever parasite (after Schaudinn, 1902).

tocysts of coelenterates. They are situated at, or symmetrically about, one pole, or in some species one at each pole. The spore contains one or more cells from which the new generation develops.

Spores of these types develop in most species from cells descended by division from a "pansporoblast," two, four, eight, or many spores developing from one pansporoblast, according to species. A definite number of cells, different in different forms, attains a polar arrangement; certain of them give rise to the polar capsules; others to the envelope, which develops great variety of form, and to one or more "germs"; or a multinucleate mass gives rise to the new individual. There is no visible indication of axiate pattern in the so-called "germ." In some forms the nuclei of cells giving rise to different parts of the spore become visibly distinguishable early in spore development. In the order Myxosporidia the cells from which spores develop arise inside an amoeboid body by delimi-

tation of a part of the cytoplasm about certain "generative" nuclei, which are thus distinguishable from other "somatic" nuclei. An example of myxosporidian spore development is given in Figure 191, with *A* showing the two spores of a pansporoblast in a developmental stage, *B* a single spore of somewhat later stage, and *C* the elongated form of the mature spore. This spore is essentially a multicellular axiate organism, and its development presents various problems. What determines the difference between generative and somatic nuclei in the multinucleate amoeboid mass, resulting

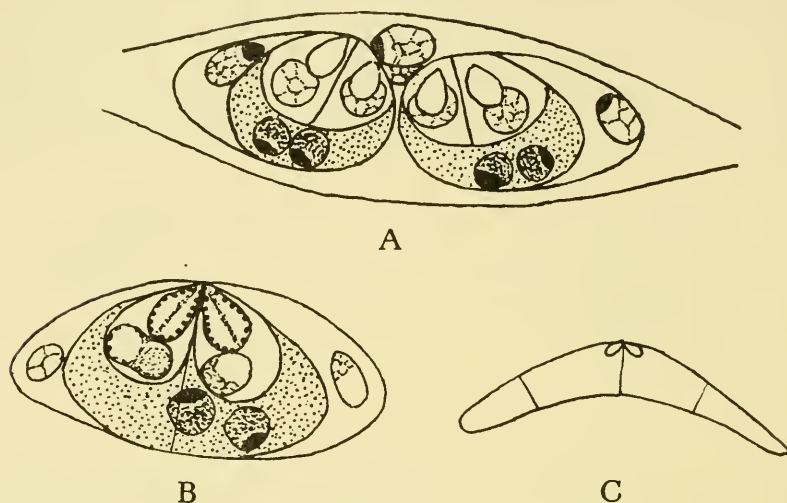


FIG. 191, *A*, *B*.—Stages of spore development of a myxosporidian, *Ceratomyxa drepanosetiae*. *A*, the two spores in amoeboid body, partially developed envelope, polar capsule cells and polar capsules outlined; spiral thread present at this stage, but not shown in figure; binucleate germ stippled. *B*, later stage of a spore, spiral threads of polar capsules indicated in section. *C*, form of mature spore (after Awerinzew, 1909).

in formation of delimited cell bodies about the former and not about the latter? What determines the axiate pattern and the differences in behavior of cells, resulting in development of polar capsules, envelope, and binucleate germ in a definite spatial pattern? These differences in fate apparently involve differentiation of capsule cells and envelope cells along quite different lines. The orientation of the two spores in Figure 191, *A*, suggests axial determination in reaction to some factor external to the pansporoblast, but various figures of this and other species show no indication of a general factor that might determine such pattern. As regards certain features of cnidosporidian development, there is difference of opinion among investigators; but there is no question concerning develop-

ment of a multicellular axiate pattern in both myxosporidian and actinosporidian spores.

The extremely minute spores of the order Microsporidia possess a single polar capsule or vacuole with long thread. Certain data suggest that in some species the axiate spore pattern may be determined by factors in the host cells, perhaps the physiological polarity of these cells, within which the spores are formed. In the genus *Curleya*, however, the pansporoblast, containing four nuclei, gives rise to four spores by elongation in four directions, the like poles of each of the four spores being, respectively, peripheral and central as regards the whole group, as if determined by the differential between surface and interior of the group.

As regards many species, there is no evidence that the "germ" of the spore possesses or retains any trace of axiate pattern in the later amoeboid stage. Some of the Myxosporidia are apparently axiate in the amoeboid stage, but whether this pattern is determined in the spore is not known. The small size and parasitic habit of these forms present difficulties to experimental control, but the many problems concerning developmental patterns in the group are no less important than those of embryonic development.³

PATTERNS OF "EMBRYONIC" OR "LARVAL" STAGES OF SUCTORIA

The Suctorina show various types of budding, and the life-cycle involves metamorphosis of a ciliated, free-swimming stage into a suctorial form, usually sessile. Some particularly interesting and puzzling questions concerning pattern arise in connection with origin and development of the buds.⁴ The ciliated stages, sometimes called "embryos"—perhaps preferably "larvae"—show in different species considerable differences in axiate and ciliary patterns and in manner of origin from the parent body. As will appear, designation of the axes is, to some extent, a matter of viewpoint. The disk of attachment of the later suctorial stage is usually visible in the free-swimming larval forms; and since this constitutes one end of the chief—often the only—axis of the suctorial form, it seems most convenient, though not necessarily otherwise significant, to regard it as representing one end of the polar or chief axis in the ciliated stage. Collin, however, regards this axis as dorsiventral.

³ It has been possible to mention only a few of the interesting features of this material. Further data appear in Doflein-Reichenow, 1929, and in the literature cited there.

⁴ The types of budding and forms of the ciliated stages are described at length in the monograph by Collin (1911, 1912), which has an extensive bibliography.

Larvae of *Podophrya fixa* are more or less ovoid in form and in transverse section, and cilia are present over the whole surface. Before attachment they become spherical and the cilia undergo regression, an equatorial band persisting longest. Another larval type is more or less discoid, circular in outline, with disk of attachment in the center of one flattened face and a marginal band of cilia (Fig. 192, A). This form may be regarded as possessing a polar pattern vertical to the plane of flattening and as completely radial. A modification of this type appears in larvae

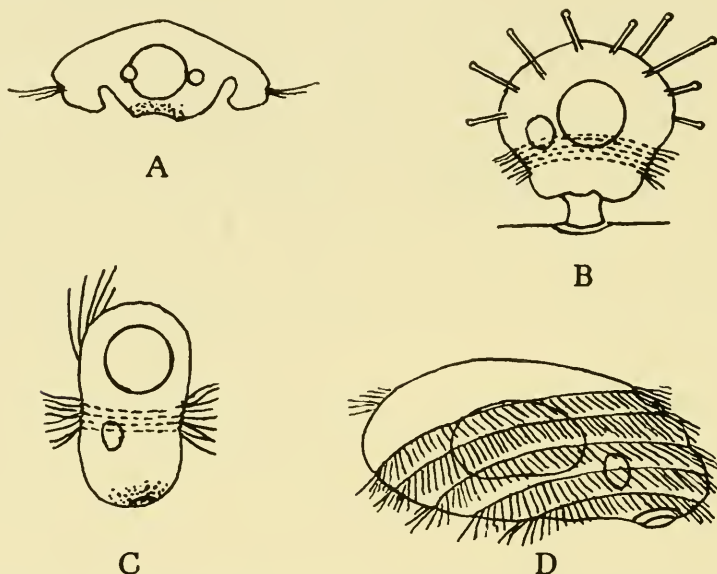


FIG. 192, A-D.—Larval forms of Suctoria. A, *Discophrya cybistri*, optical section, showing granules about disk of attachment, meganucleus, two vacuoles, and ciliary band; B, *Tokophrya cyclopum*, mature attached stage; C, “larval” stage of B, showing disk of attachment with granules, cilia, meganucleus, and one vacuole; D, *Acineta tuberosa*, with chief axis obliquely anteroposterior (after Collin, 1912).

elongated vertically to this polar axis and therefore possessing a physiological anteroposterior pattern and a bilaterality, the original polar axis being physiologically dorsiventral with the disk of attachment ventral. According to Collin, the physiologically posterior end of the larva is the end of the bud last detached from the parent. These larvae usually move with the “ventral” surface in contact with a substrate. In larvae of certain other species the disk of attachment is at one pole of a longitudinal axis, and this pole is anterior in locomotion. These forms possess an equatorial ciliary band consisting of rows of cilia, different in number in different species, and a group of cilia at one side of the pole opposite the disk of

attachment. Except for this group of cilia the larvae appear to be completely radial (Fig. 192, *B, C*). Larvae of this type are free-swimming. In still other larval forms the original polar axis, indicated by the disk of attachment, is oblique to a dorsiventral pattern; consequently, bilaterality is present, but a different bilaterality from that mentioned above (Fig. 192, *D*), in that the polar axis tends to become physiologically anteroposterior while in the other type it is physiologically dorsiventral and

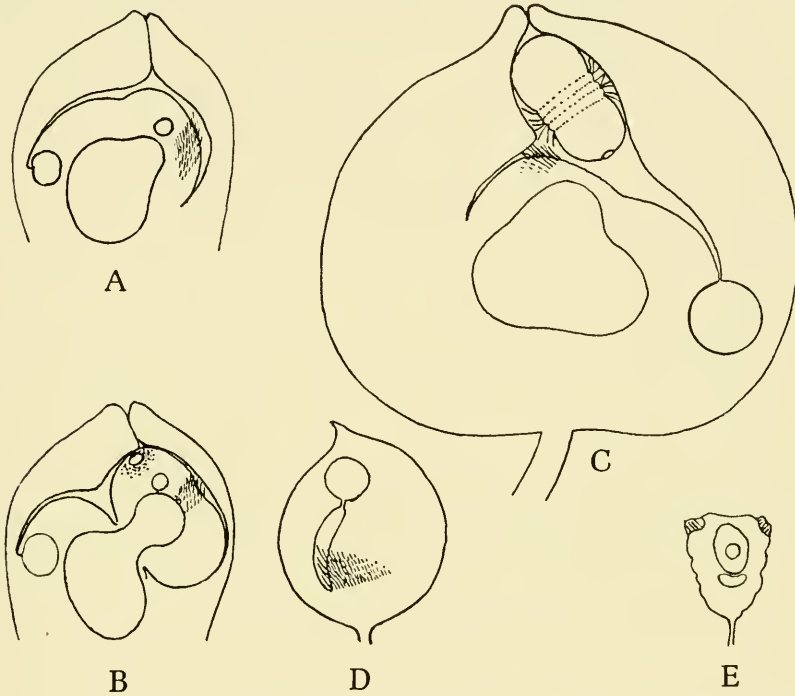


FIG. 193, *A-E*.—Larval development from internal buds in Suctoria. *A, B*, two stages of bud development in *Tokophrya cyclopum*, dividing meganucleus in *B*.; *C*, *Choanophrya infundibulifera* with one larva free in cavity, a second developing, and perhaps a third in still earlier stage; *D*, early stage of *Pseudogemma fraiponti*; *E*, *Acineta tuberosa* (after Collin, 1912).

at right angles to the anteroposterior axis. As regards the variety of axial relations, these larval forms resemble somewhat the types of axiate pattern in echinoderms.

Origin and development of the larval forms from the suctorial form differ in different species and in some seem to differ from anything known in other groups. Larvae of many species develop from "internal buds" in a cavity of the parent body (Fig. 193). According to various authors, their development in certain species shows two features of particular in-

terest. First, the polar (longitudinal) axis of the larva has been repeatedly described and figured as transverse to the chief axis of the parent suctorial body; second, separation and differentiation of the larval from the parent body progresses transversely to the larval longitudinal axis. Two stages of this type of larval development are shown in Figure 193, *A* and *B*. In *A* the larval axis is almost parallel to the parental axis, and in *B* it is not transverse. According to Collin, this position is merely an anomaly, but it suggests the possibility that the larval axis may be primarily parallel to, and determined by, the parental axis and that the transverse position, even though it may be assumed or approached very early in development, is actually secondary. If this is true, the larval pole bearing the disk of attachment develops toward the distal pole of the parent; and if a gradient is present in the parent body, the distal pole probably represents its high end. As already noted, the larval pole of attachment is in advance in locomotion, that is, it is physiologically apical. In Figure 193, *C*, of another genus, the position of the fully developed larva with the pole of attachment more or less proximally directed with respect to the parent has doubtless resulted from locomotion after separation. The axis of the second developing larva is oblique, not transverse to the parental axis; and the figure suggests that a third larva may be developing below and to the right of the second, with its axis still more nearly in line with the parental axis. The progress of development transversely in the larva is indicated in Figure 193, *A*, *B*, and *C*; but in *A* and *B* and in the second larva of *C* the physiologically apical pole of attachment appears to be slightly in advance of the opposite pole as regards separation from the parent body, that is, there is some evidence of an apicobasal differential. Collin gives figures of other species showing the larval axis parallel or almost parallel to the parental axis (Fig. 193, *D*, *E*).⁵ However, even if the larval polar axis is primarily coincident in direction with, and a part of, the parental axis, the question of the factors determining the change to transverse position in certain species and the plane in which it occurs remains. If it is primarily transverse to the polar axis, as it appears to be from many descriptions and figures of certain species, the manner of its establishment and the progress of larval development from one side of the body to the other present puzzling problems.

Larvae of some Suctoria develop from external buds. The larva of

⁵ See also Collin, Figs. III *f*, LXXXIII, LXXXIV, XCI *a*, XCV *e*. Still other figures suggest that the polar axis of the larval type of Fig. 192, *D*, may also be primarily parallel to the parental axis.

P. fixa, for example, develops from the apical region of the parent, the cilia covering its whole body replacing the suckorial tentacles progressively from the free end proximally (Fig. 194, *A*). Larval development from an external bud in a manner like the development from internal buds shown in Figure 193, *A-D*, has also been observed in some species (Fig. 194, *B*) and again raises the question of relation of bud axis and parental axis. According to Collin, the bud axis in these forms is usually transverse to the parental axis but may be oblique, as in Figure 194, *B*. This figure suggests, however, that the bud axis may have been primarily parallel to the parental axis.

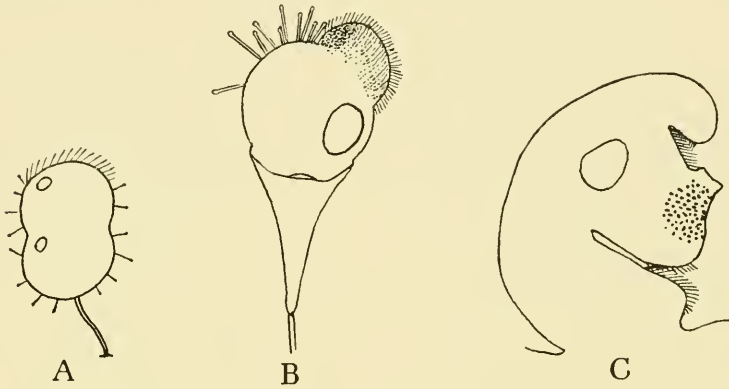


FIG. 194, *A-C*.—Larval development from external buds in Suctoria. *A*, *Podophrya*; *B*, *Paracinetia patula*; *C*, *Ephestia gemmipara* in sagittal section, showing invaginated ciliary band near free end, "cytostome" near attached end, and pole of attachment indicated by secretory granules (after Collin, 1912).

Larvae of some other species develop from external buds arising in a circle about the periphery of the more or less flattened distal or apical surface of the suckorial form. In a typical case the surface of the bud toward the center of the parental disk becomes flattened; the disk of attachment and rows of cilia develop on it, the ciliated region becoming invaginated; and an infolding, regarded by Collin as a rudimentary cytostome, also appears near the attached end of the larva (Fig. 194, *C*). In such larvae the polar axis, indicated by the disk of attachment, either has become physiologically dorsiventral, and is transverse or oblique to the suckorial parental axis, or is bent in a right angle. The larva shows an anteroposterior pattern parallel to the parental axis. It is not evident from the descriptions and figures how this pattern originates, but the following suggestion may possibly have some value. Assuming that the

pole of attachment of the larva, indicated by stippling in Figure 194, C, was originally determined in the apical region of the early bud, a bending or folding-over of the growing bud toward the center of the parental disk would result in the position and form of Figure 194, C. Larval form and invagination of the ciliated band suggest that such a bending or folding by differential growth of the two sides may have taken place. The "cytostome," which apparently has no function and disappears later, may also result from a bending or folding of the larval body in early stages. According to this suggestion, the original polar axis of the larva is primarily

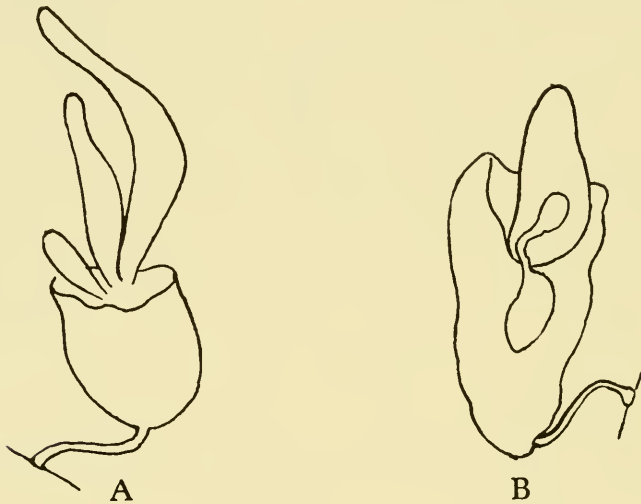


FIG. 195, A, B.—*Ophryodendron reversum*; early stages of vermiform individuals developing from buds; in B, dividing meganucleus (after Collin, 1912).

parallel to, and presumably determined by, either the parent axis or a local bud activation and becomes bent so that the disk of attachment is physiologically ventral, perhaps in consequence of a central-peripheral radial differential in the parental distal region. The peripheral position of the buds suggests that such a differential is present. In short, even the larval pattern in these species may perhaps be determined by the relation of the bud to the parent body.

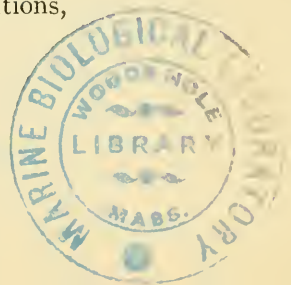
Certain suctorial species give rise, by external budding, to extremely elongated vermiform individuals (Fig. 195) with longitudinal axes parallel to the parental axis and apparently determined by it or resulting from a local activation. Species of the genus *Hypocoma*, ectoparasitic on other protozoa, and some other forms develop only a single suctorial tentacle

and apparently have no attached stage. They show a physiological and morphological dorsiventrality with ventral ciliary bands in elliptical pattern, except for slight lateral asymmetry. These forms undergo transverse fission like most ciliates.

At present we have only the data of observation concerning these patterns of suctorial development. The peculiar development of axiate larval patterns from internal buds, with progress of differentiation from one side to the other, differs from anything known in other groups. The pole of attachment is physiologically ventral in some forms (Fig. 192, *A*), physiologically apical in others (Fig. 192, *C*), and physiologically almost anterior in still others (Fig. 192, *D*).

CERTAIN FEATURES OF PATTERN IN OTHER AXIATE PROTOZOA

Axiate patterns of individual cells composing the spherical colonial flagellates, such as *Pandorina*, *Volvox*, and various others, appear to be directly related to a surface-interior difference; but they may represent polarities persisting through the successive divisions from the primary cell of the colony, the divisions being parallel to the polar axes of the cells. A relation between cell formation and pattern, apparently similar to that so general among Sporozoa, appears in certain reproductions of Mastigophora. Swarm-spore development in *Noctiluca* is preceded by repeated nuclear division near the surface of a part of the parent body, the apical region. Following this period, budding of a cell body in relation to each nucleus results in development of a swarm spore or perhaps a gamete with flagella originating from the apex of the bud (Pratje, 1921). The surface-interior differential of the mother cell evidently plays a part in determining the position of the nuclei and may be concerned, together with the budding, in determining the axiate pattern of the spore. The longitudinal fission so generally characteristic of Mastigophora may be regarded as a sort of budding of the two cells from each other, with polarity persisting but with reconstitution of symmetry or asymmetry in each part in definite relation to the polar pattern as separation proceeds. The transverse fissions characteristic of most ciliates involve, of course, polar reconstitution. In many ciliates, however, extensive dedifferentiation of the pre-existing pattern is associated with fission, so that what finally results is actually a new pattern, but one that develops in direct relation to the original pattern and presumably under its influence. For example, the two new cirrus fields appearing in fissions, as well as in reconstitutions,



of *Hypotricha* represent a new pattern appearing in a certain definite relation to the parent pattern.⁶

In *Vorticella* the two products of the longitudinal fission differ. One remains attached to the stalk and develops peristome and gullet. In the other a ciliary ring develops about the originally basal pole, the individual becomes free, swims with the originally basal pole in advance, and later attaches by this pole; following attachment the ciliary ring disappears, and the peristomial region develops. The question whether this developmental history involves two reversals of polarity and of dominance arises from the apparently opposed character of the two patterns. If one product of fission in *Vorticella* is less directly connected with the stalk than the other and if reduction of the peristome permits development of the basal ciliary ring and attainment of dominance by the basal region in this product, its separation would be expected. As in the planulae of calyptoblast hydroids (p. 96) and some larvae of Suctoria (p. 612), the pole functionally apical in the free-swimming stage becomes basal on attachment. Both products of fission usually remain attached in the colonial vorticellids, but certain zooids may become free-swimming "ciliospores" with change in pattern and behavior very similar to that in the free form of *Vorticella*. The free-swimming microgametes, resulting from successive fissions without intervening peristome development, also show similar change in pattern and behavior. Evidently this "reversal" represents a characteristic form of development for this genus and, to some extent, for other members of the group.

SYMMETRIES AND ASYMMETRIES OF PROTOZOA

A high degree of morphological differentiation and an almost endless variety of symmetries and asymmetries, highly species-specific in character, appear in the ectoplasm of flagellate and ciliate protozoa.⁷ Some of the varieties of larval suctorial pattern have already been described. In some forms the pattern is apparently strictly radial—for example, *Prorodon*—but the body revolves about the polar axis in locomotion like free-swimming developmental stages of many invertebrates, indicating

⁶ Wallengren, 1901; D. B. Young, 1922; Dembowska, 1925, 1926; Taylor, 1928.

⁷ Figures are not given: first, because only a very large number would give any adequate survey of the variety of these morphological patterns; second, because every student of zoölogy is familiar with some of them, and some are figured and described in every textbook of zoölogy and many others in the works on protozoa—for example, Kent, 1880–82; Bütschli, 1883–89; Doflein-Reichenow, 1929; Calkins, 1933, etc.; also a voluminous literature on particular species of groups.

a spiral pattern of ciliary beat. Very commonly, however, the morphological pattern shows a spiral asymmetry, even in forms such as *Paramecium*, in which there is comparatively little regional morphological differentiation; and spiral pattern is often much more conspicuous in the more highly differentiated forms. The pattern of the peristomial cilia and membranelles of ciliates is usually spiral, and in many species the whole ectoplasm apparently possesses spiral pattern. Among the parasitic flagellates remarkable developments of certain types of spiral pattern appear. In the genus *Spirotrichonympha*, for example, spiral bands at or near the inner surface of the ectoplasm make a series of definite, regularly spaced turns around the body, and the numerous flagella connected with them form corresponding spirals. Of two species, *S. polygyra* and *S. bispira*, recently described by Cleveland (1938) with spiral bands extending over some three-fourths of the body length, the former shows forty-five, the latter thirty-four, turns of the bands; and, according to Cleveland's figures, there is little deviation from geometrical regularity in the spacing of successive turns and slope of spiral. In division of *S. polygyra* the bands unwind, beginning at the anterior end, two going to each daughter individual and two developing anew. In *S. bispira* a new band without spiral arrangement develops from the point of origin of the parent bands at the anterior end and migrates to the posterior end of the cell, which becomes the anterior end of one of the daughter individuals. There the band gradually becomes spiral, at first not completely regular, beginning at the end originally anterior in the parent cell and now at the new anterior end of the daughter individual. The second flagellar band develops from the anterior end of the first, extends posteriorly in the new individual, and forms a spiral between the turns of the first band. Various other features of pattern in these species—the axostyle and the elongated centrioles—are of interest as indicating a high degree of differentiation, but the description of these remarkable forms and their divisions serves to emphasize the fact that we have not the remotest conception of the physiological factors concerned in the origin and development of such pattern, but that it originates and is localized in relation to regional differences in physiological activity with a definite pattern seems evident.

A dorsiventral, as well as anteroposterior, pattern appears in the *Hypotricha*, together with a great variety of morphological asymmetries in arrangement of cirri and membranelles, most of which are genus- and species-specific; but in cell division the asymmetries of daughter individ-

uals originate as ectoplasmic fields in definite relation to the parental pattern, and their orientation is presumably determined by it.

In many of these protozoan forms more or less morphological dedifferentiation occurs in connection with division, and the characteristic asymmetries redevelop in the new cell generation; but the essential physiological factors of pattern evidently persist, or originate within the parental pattern in an orientation with respect to it which is definite and characteristic for the species. That the various asymmetries are in some way associated with the specifically different physicochemical constitutions of the species-protoplasms seems beyond question. Various suggestions and possibilities will be considered in a later section after some other asymmetries of unicellular individuals have been noted.

PATTERNS OF PLANT AND ANIMAL SPERMS

Antherozoids, and spermatozoids of plants and spermatozoa of animals show great variety of morphological pattern. A heteropolar longitudinal pattern is almost always present; in some forms there is complete, or almost complete, radial symmetry about the axis of this pattern; in others a spiral pattern develops, either in the whole individual or in some part of it; and in still others other asymmetries characteristic for the species appear.

ANTHEROZOIDS AND SPERMATOZOIDS OF PLANTS

The sperms of many algae are biflagellate forms similar to the zoospores in general pattern (see Fig. 186, *A*, *C*) but often smaller than zoospores of the same species. In some forms there appear to be gradations in size and behavior as zoospores or gametes. The pigmented spot or stigma is asymmetrical in position in forms with terminal flagella, as in similar zoospores (Fig. 186, *A*); and in forms with lateral flagella both flagella and stigma are asymmetrical in position (Fig. 186, *C*). As in the case of zoospores, the question how these patterns originate or whether they show relation to any particular factors is unanswered (see pp. 601-3).

The antherozoid or spermatozoid of *Chara* is an elongated, spirally coiled biflagellate cell (Fig. 196, *A*). The antheridial filament consists of a single series of flattened cells, each of which metamorphoses into a single antherozoid. After the final division the nucleus moves toward the side wall; the blepharoplast appears adjoining the cell surface toward the base of the filament, elongates, and becomes spirally coiled; and elongation and spiral coiling of the nucleus follow, the axis about which the spiral develops

coinciding with the axis of the antheridial filament.⁸ In this development several points of interest as regards pattern appear. As regards direction

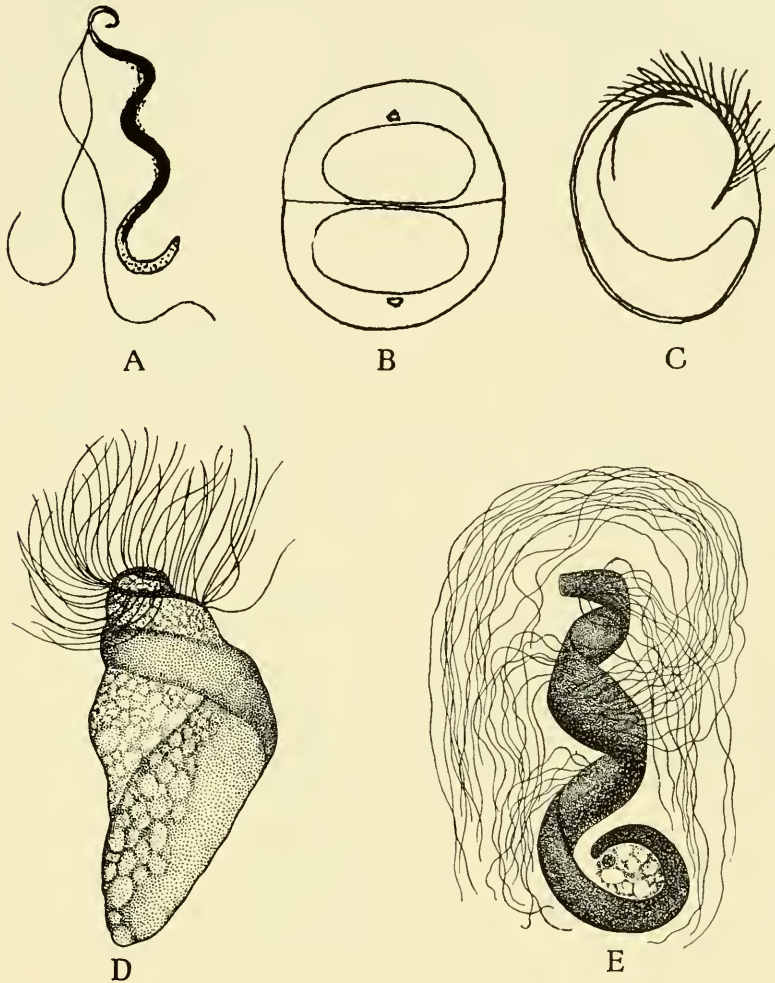


FIG. 196, A-E.—Plant spermatozoids. A, *Chara* (after Belajeff, 1894); B, C, D, stages in development of *Equisetum* spermatozoid (after Sharp, 1912); E, spermatozoid of fern, *Nephrodium* (from Yamanouchi, 1908).

of nuclear movement toward one side of the cell, both Belajeff and Mottier give figures in which nuclei of successive cells of a filament have evidently moved in the same direction, as if reacting to some factor external to the

⁸ Belajeff, 1894; Mottier, 1904.

filament, and also figures in which the two plane walls of successive filament cells diverge toward the same side and the nuclei are on the side where the walls are farthest apart. The blepharoplast makes its appearance adjoining the transverse cell wall toward the base of the filament, and spermatozoids develop with the flagellate end also toward the base, suggesting orientation of the pattern in relation to some factor in the filament as a whole, perhaps a gradient of some sort.

Spiral spermatozoid pattern is characteristic of bryophytes and pteridophytes and develops as elongation and spiral coiling, first of the blepharoplast, then of the nucleus. In certain of these forms what may be called

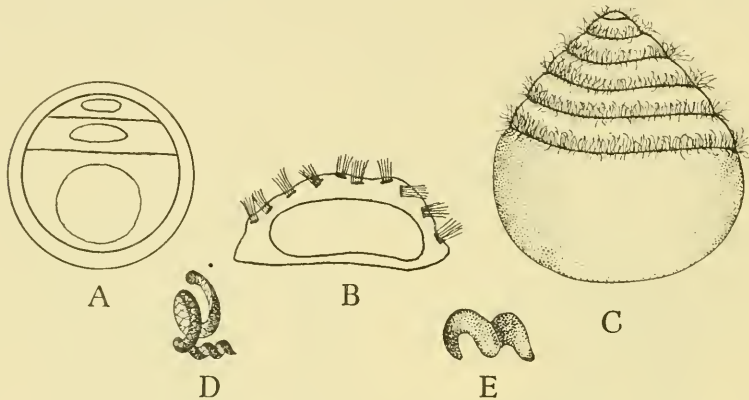


FIG. 197, A-E.—Male gametes of plants. A, pollen grain; B, section of developing spermatozoid of *Cycas revoluta* (after Ikeno, 1898); C, spermatozoid of the cycad *Zamia* (from Webber, 1901); D, male nucleus of the sunflower *Helianthus* (from Nawaschin, 1900); E, male nucleus of *Silphium* (from Merrell, 1900).

the “polar axis,” that is, the axis about which the spiral coiling occurs, coincides in direction with the axis of the final mitotic spindle, the blepharoplast before elongation being at the pole of the spindle. For example, in *Equisetum* (Sharp, 1912) the two sister spermatids of Figure 196, B, evidently possess an axiate pattern in relation to each other, and the later spiral coiling of blepharoplast and nucleus is about this axis (Fig. 196, C), resulting in the spermatozoid (Fig. 196, D). Spermatozoid development in the fern *Nephrodium* (Fig. 196, E) is apparently very similar (Yamanouchi, 1908). In the cycads—a group related, on the one hand, to pteridophytes and, on the other, to spermatophytes—spirally ciliated spermatozoids also develop. The cycad pollen grain (Fig. 197, A) shows a definite axiate pattern, consisting of three cells, two representing the

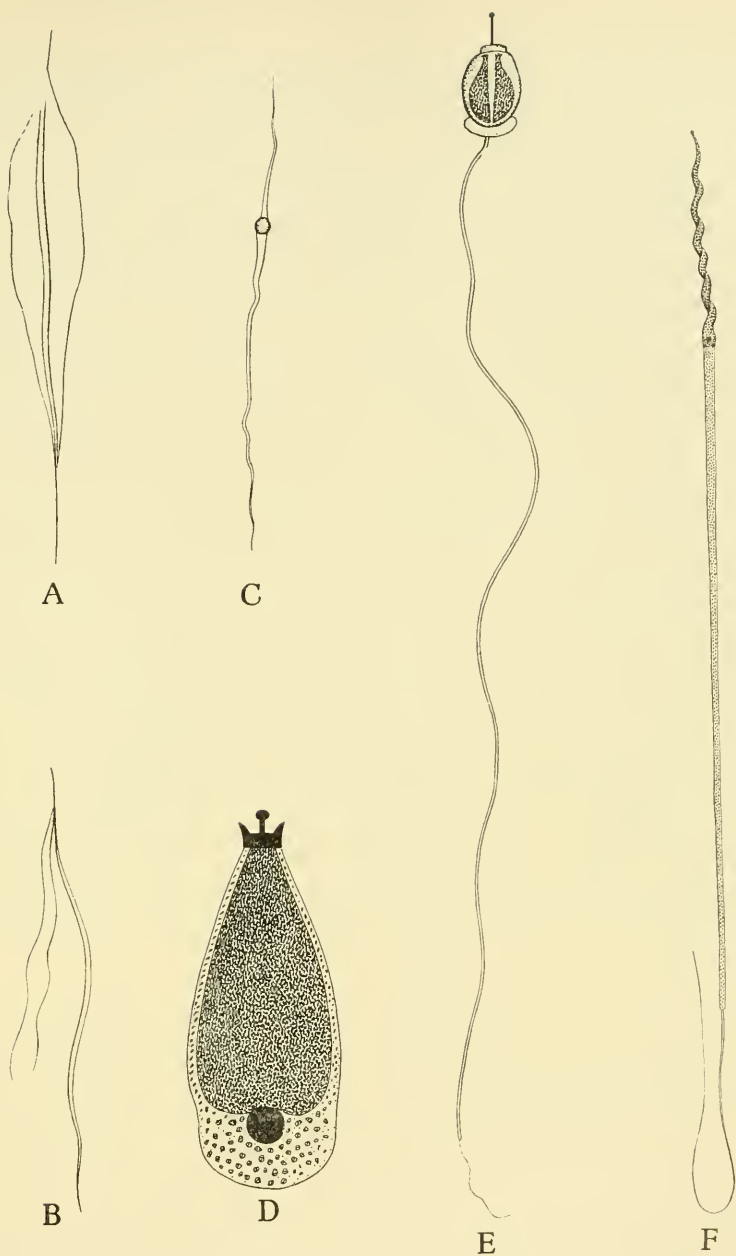


FIG. 198, A-F.—Animal spermatozoa. A, *Plagiostomum*, a turbellarian (from Böhmig, 1891); B, *Castroda* and C, *Mesostomum*, both turbellaria (from Luther, 1904); D, *Ascaris megalocephala*, a nematode (from Scheben, 1905); E, *Nereis*, a polychete annelid (from F. R. Lillie, 1912); F, *Paludina*, a gastropod (from Meves, 1903).

male gametophyte, the third giving rise to spermatozoids. This pattern is presumably determined in the microsporangium. The polarity of the grain is also evident in its germination and formation of the pollen tube at the small-celled pole. In the tube the generative nucleus divides with spindle transverse to the long axis of the tube, forming two hemispherical cells. In each of these the blepharoplast, described as derived from the centrosome, elongates, becomes spirally coiled anticlockwise, and gives rise to a band of cilia (Fig. 197, *B, C*). The axis of coiling coincides with the spindle axis of the preceding division; the ciliated part of the spermatozoid develops from the region about the pole of the last spindle; and the rounded, nonciliated part from the flattened cell surface in contact with the sister cell.⁹ The spermatozoid axis is at right angles to the axis of the pollen grain and pollen tube and is apparently determined by some factor correlated with division of the spermatogenous cell, since it coincides with the axis of the spindle and is indicated by the hemispherical form of the spermatid and by position of nucleus and centrosome; and this division is definitely related to the axis of the pollen grain and tube. In short, each step in development of the polar pattern appears in a definite relation to a pre-existing pattern. The spiral of the blepharoplast appears only secondarily and in a definite relation to the polar axis. During early development of the spiral band a beaklike extension of the nucleus becomes closely associated with the blepharoplast, as if it were about to elongate and become spiral, as in the spermatozoids described above; but this association is only temporary, and the nucleus does not become spiral. The male nuclei of various seed plants, however, do develop a more or less pronounced spiral form preceding fertilization (Fig. 197, *D, E*).

ANIMAL SPERMATOOA

The great variety of form, the spirals, and specific asymmetries of animal spermatozoa have long been familiar to students of spermatogenesis. The spermatozoa of Figures 198-200 show fully developed spermatozoa of turbellaria (Fig. 198, *A-C*), a nematode (Fig. 198, *D*), a gasteropod (Fig. 198, *E*), an annelid (Fig. 198, *F*), the more or less radial spermatozoa occurring among Crustacea (Fig. 199, *A-C*), a spider (Fig. 199, *D*), a beetle (Fig. 199, *E*), a fish (Fig. 200, *A*), a salamander (Fig. 200, *B*), and a toad, with undulating membrane, resembling somewhat that of a trypanosome (Fig. 200, *C*). In certain entomostraca the spermatozoa are spherical cells; those of some others are amoeboid, according to Weismann

⁹ Ikeno, 1898; Webber, 1901.

(1880) and Zacharias (1885); and those of the cirripeds are described as fibrillar without differentiation of head or tail.¹⁰

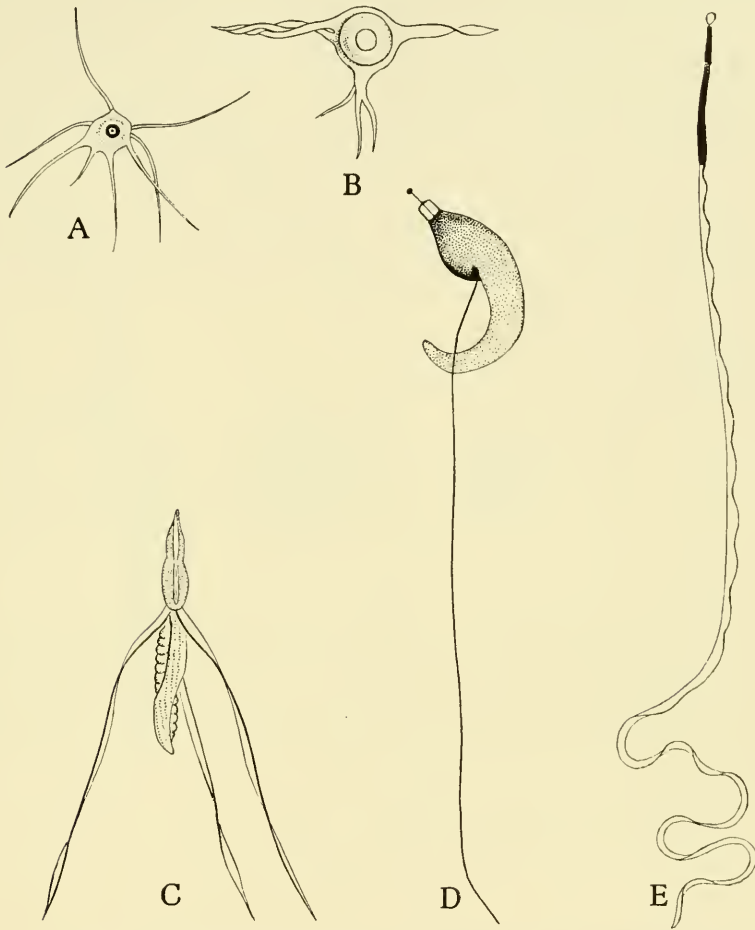


FIG. 199, A-E.—Arthropod spermatozoa. A-C, *Pinnotheres*, *Maja*, *Munidia*, all crustacea (from Koltzoff, 1906); D, *Agalena*, an arachnid (from Bösenberg, 1905); E, *Copris*, a beetle (from Ballowitz, 1890b).

Most animal spermatozoa are distinctly axiate, and spiral patterns and specific asymmetries are related in a definite way to the axiate pattern. Moreover, in many forms the polar pattern appears to be definitely re-

¹⁰ For further descriptions and figures of spermatozoa see Retzius, 1881; many papers by Ballowitz, 1886-1908; Koltzoff, 1906, 1909; Korschelt und Heider, 1902, and literature cited; E. B. Wilson, 1925, and literature cited.

lated in the testis to a blastophore or cytophore or a particular kind of accessory cell. The cytophore, characteristic of many invertebrates, has somewhat different origin in different species. Successive divisions of



FIG. 200, A-C.—Fish and amphibian spermatozoa. *A*, *Raja*; *B*, *Triton* (*A* and *B* from Ballowitz, 1890c); *C*, *Bombinator* (from Broman, 1900).

spermatogonia and spermatocytes are cytoplasmically incomplete in some forms; and more or less spherical groups of cells, separate peripherally, continuous centrally, result, the central cytoplasm becoming the cytophore (Fig. 201, *A*). In other species the cytophore develops from degenerated cells in the center of a mass (Fig. 201, *B*). The spermatozoa de-

velop, either irregularly scattered over the surface of the cytophore (Fig. 201, *C*) or covering the whole surface like an epithelium (Fig. 201, *D*). The point of particular interest is that the polar pattern of the spermatozoon develops radially to the cytophore, the motile tail, when present, being peripheral (Fig. 201, *C*, *D*). The tubular nematode testis contains a rhachis to which the cells remain attached up to a late stage of sperm development, and the sperm axis is apparently the axis between free and attached pole of the spermatocyte.

Spermatozoa of many other forms, both invertebrate and vertebrate, develop in bundles with axes parallel and heads imbedded in, or in contact with, certain accessory cells. The spermatocysts, common among insects

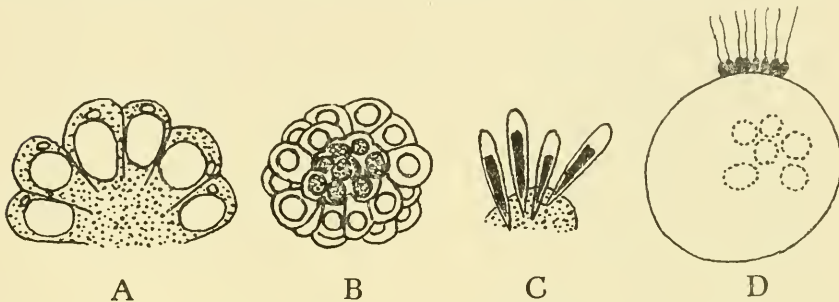


FIG. 201, *A-D*.—Sperm development in relation to a cytophore. *A*, spermatocytes and part of cytoplasmic cytophore of earthworm (after Calkins, 1895); *B*, origin of cytophore of the opisthobranch, *Triopa clavigera*, from degenerating cells (after O. S. Jensen, 1883); *C*, part of cytophore and developing spermatozoa of the turbellarian, *Plagiostomum sulphureum* (after Böhmig, 1891); *D*, cytophore of the annelid, *Clitellio arenarius*, containing degenerating nuclei and showing a few of the spermatozoa which cover the whole surface (after O. S. Jensen, 1883).

and the lower vertebrates, consist of spermatogenous cells inclosed in an epithelial cyst; and spermatozoa develop parallel with heads toward, or imbedded in, one of the cells of the cyst wall, usually of considerable size, and believed to show evidence of secretory activity. How this particular cell is determined does not appear. The relation of spermatid pattern to the basal or Sertoli cells appears very clearly in the mammalian testis (Fig. 202, *A*, *B*).

Cytophores and accessory cells to which the sperm axes are definitely related have commonly been regarded as nutritive in function; and cases like Figure 202, *A* and *B*, have suggested the possibility of a tactic or tropistic reaction of the spermatid to the Sertoli cell. But the question arises whether the observed relations represent orientation of an axiate pattern already determined, that is, a tropism in the strict sense, or de-

termination of axiate pattern by the relation to the cytophore or accessory cell (see Broman, 1902). It seems probable that in at least some forms with cytophore the axiate pattern of the spermatozoon is determined by the difference in conditions between the pole toward the cytophore and

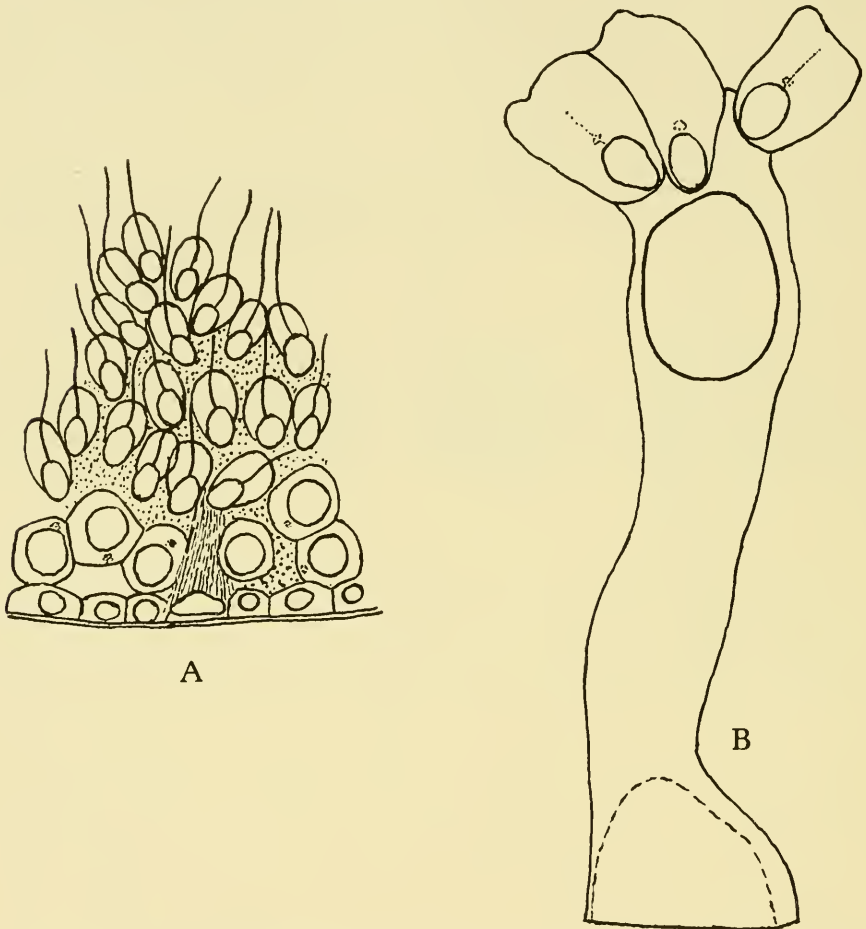


FIG. 202, *A*, *B*.—Relation of mammalian spermatids to Sertoli cells. *A*, rat (after von Lenhossek, 1898); *B*, man (after Broman, 1902).

the peripheral pole. In many cases there is cytoplasmic continuity with the cytophore until development of pattern is far advanced. A tropistic reaction is, of course, possible in the cases of secondary association with an accessory cell, as in Figure 202, *A* and *B*, but does not appear very probable. The polar position of the nuclei suggests a reaction to a factor

external to the spermatid and certainly shows a definite relation to the accessory cell, as does the axial fiber.

Studies of spermatogenesis have usually been concerned with the sperm cell alone and have given little attention to the question of possible determination of its polar pattern by factors external to it. However, in many accounts of spermatogenesis extensive changes in position of centriole, acroblast, etc., are described.¹¹ Axiate pattern of the spermatozoon in many forms develops after the final division by changes in relative position (sometimes of almost 180°) of distinguishable parts of the cell. If the pattern is inherent in the cell, these shifts in position seem difficult to account for, and they have usually been merely described without any attempt at interpretation. But if they represent reaction of different cell constituents to a differential or other factor external to the cell, the difficulty of interpretation is certainly lessened.

Cytophores or other accessory cells are entirely absent from spermatogenesis of some forms; nevertheless, a spermatozoan pattern develops. Cytological studies suggest that pattern may be determined by the final cell division, as it apparently is in certain plant spermatozoids, the centrosome-nucleus axis determined by direction of division becoming the spermatozoan axis, with the tail, when present, developing from the centrosomal pole. This axis probably becomes the sperm axis unless an axis in different direction is determined by reaction to some external factor, such as cytophore or accessory cell.

UNICELLULAR ASYMMETRIES: QUESTIONS AND SUGGESTIONS

Development of a polar axiate pattern in animal spermatozoa apparently precedes appearance of spirals and specific asymmetries. This seems also to be true for plant spermatozoids with spiral patterns, but in those the primary pattern is usually merely that resulting from the final cell division. In general, the spiral patterns and specific asymmetries seem to develop secondarily in a definite orientation with respect to the primary pattern. Moreover, they appear to be associated with a high degree of morphological structurization and differentiation rather than to represent a general property of protoplasts. As regards the protozoan asymmetries, it may appear at first glance that there is no such distinction between a primary axiate pattern and secondary asymmetries. In protozoan fission the patterns of the daughter individuals are not entirely

¹¹ For figures and description of cases see E. B. Wilson, 1925, pp. 356-85. Many others are given in the literature of spermatogenesis.

new patterns but are reconstitutions from portions of the parent pattern or in certain orientation with respect to it. In the longitudinal fissions of flagellates the spirals and asymmetries are apparently remade in relation to a polar pattern. Divisions of the two species of *Spirotrichonympha*, as described by Cleveland, provide interesting evidence on this point (see p. 617). In *S. polygyra* the four spiral bands unwind, two of them form a new spiral about each new polar axis, and two new bands develop about each axis. In *S. bispira* a new band begins to develop in the parental anterior region, migrates posteriorly, and becomes spiral only after it reaches the original posterior end, which becomes a new anterior end. Moreover, when the spirals appear, they are at first irregular and only gradually attain the regularity characteristic of the fully developed animal. Cleveland's description suggests that the new spiral patterns are determined by a more general longitudinal pattern already present in the new anterior regions. When *Stentor* divides, the new peristomial band is not at first spiral and only gradually attains its definitive form, apparently in relation to an axiate pattern already present. According to a personal communication from Dr. C. V. Taylor, the ciliate *Colpoda duodenaria* at the beginning of excystment is almost completely radial, as far as visible pattern is concerned. The ends of the ciliary meridians center about the two poles with only slight indication of the spiral asymmetry characteristic of the fully differentiated individual. The oral region begins development as a shallow invagination near, and at one side of, the anterior pole and progresses posteriorly in a slight spiral. In association with this spiral invagination of the mouth the anterior portions of the ciliary meridians undergo spiral twisting. As the oral region migrates posteriorly, the anterior polar region changes its position correspondingly, with flexure of the polar axis, so that the original anterior pole comes eventually to lie somewhat anterior to the mouth, but distinctly lateral as regards the whole body. At the posterior end, however, the radial arrangement of the ciliary meridians persists. Spiral pattern in this species is apparently almost completely obliterated in the encysted stage and reconstitutes in relation to a polar pattern on excystment.

Division in many forms, both flagellates and ciliates, involves what seems to be more or less dedifferentiation of parental organs and development of new organs, either in normal relation to each other or, in case of the cirri of hypotrichs, in localized areas with a following change in position, apparently in relation to a pattern.

In short, various lines of evidence indicate that in the protozoa, as

well as in spermatozoa, the spiral features of pattern and the other specific asymmetries develop secondarily in a definite relation to a primary pattern. In this connection it may be recalled that presence of a longitudinal physiological gradient has been demonstrated in the ectoplasm of a considerable number of ciliates by various methods, and at present there is no evidence to indicate that the differentials in the protoplasmic substrate and its activities which constitute these gradients are not the primary axiate pattern. If they are primary, the spirals and asymmetries represent secondary structural expressions and differentiations of the primary pattern in the various species-protoplasms. Whether axiate pattern of the spermatid is based on a gradient pattern is not known, but the apparent determination of the spermatid polar axis by an external differential in many forms and the changes in position of parts of the cell suggest that a gradient pattern may be present.

Whether or not the primary pattern in these unicellular individuals is a gradient, the problem of the nature of the spirals and other asymmetries and their relation to it remains. The possibility suggests itself that many of these features of unicellular pattern may result from, or be expressions of, molecular or micellar structure or aggregation in definite orientation. Researches of recent years with polarized light and X-rays have given evidence of orientation of molecules or particles in cellulose membranes and fibers, various animal fibrillar structures, connective tissue, muscle, nerve, keratin of hairs and feathers, chitin, etc. Thus far this evidence of molecular pattern in morphological structure concerns chiefly highly differentiated and structurized protoplasms, proteins, keratin, cellulose, and other nonliving products of protoplasmic activity rather than protoplasms in general. Various fibrillar structures appear in protoplasms under various conditions, but many of these are temporary and disappear completely when conditions change. Granting that proteins, cellulose, and other organic substances are, or may become, indefinitely long chains of chemical groups with definite polarity and symmetry or asymmetry, that these chains may aggregate with definite orientation into larger units (crystallites) which give evidence of crystalline structure, and that these crystallites may also undergo further aggregation with definite orientation, there is, at present, no evidence that a definite and persistent structure of this kind is a primary and fundamental property of protoplasms. It appears rather to be a feature of differentiation and structurization. If this is the case, the definite orientation of molecules or chains is probably not autonomous but a reaction to something; and since these orienta-

tions apparently originate in the course of development and differentiation, they are presumably reactions to some factor of developmental pattern. Such a factor may be local and related to cell surfaces, as the cellulose pattern evidently is in various cells, or to local tensions or pressures in a tissue, as in connective tissue, or within the cell, as perhaps in development of the mitotic figure; or it may be associated with organismic pattern, particularly if that pattern is unicellular. In a multicellular pattern local conditions may be more effective in determining molecular or micellar pattern than the general pattern of the whole, though the local factors are doubtless derivatives of the general pattern.

Most spermatozoa are certainly highly differentiated and structurized unicellular organisms and contain little or nothing resembling even the protoplasts of ordinary tissue cells, and still less those of embryonic cells. If molecular pattern and orientation can become evident in morphological pattern in any cells, it seems likely to be in spermatozoa. They are minute and undergo perhaps a more extreme structural differentiation than any other living cells. Many sperm heads and tails of some sperms are more or less birefringent, but spherical sperm heads are apparently not birefringent.¹² According to recent X-ray work, some sperm heads show characteristics of fluid crystalline state. Separation of spermatozoan tails into parallel fibrillae by treatment with various agents is also of interest here, since it suggests a parallel orientation of elongated units (Ballowitz, 1890a, *b*; 1895). As already pointed out, however, earlier stages of spermatozoan development show only a general axiate pattern, presumably involving the cell metabolism and perhaps primarily nothing more than a gradient.

Although the ectoplasm of flagellates and ciliates appears to be less extremely differentiated than many spermatozoa, it, or certain parts of it, certainly undergo high degrees of structurization; and this is apparently most extreme in forms with the most extreme specific spirals or other asymmetries. The frequency of spiral patterns in spermatozoa and axiate Protozoa and various other organisms is of particular interest. They probably represent the reaction of many protoplasts to presence of an axiate pattern, but we still lack definite information as to their origin and nature. Except that they are spiral, they differ widely and specifically in different species. The differences do not seem to have any fundamental or necessary relation to the life of the individual. Corkscrew heads or spirally twisted tails may be present or absent in spermatozoa,

¹² W. J. Schmidt, 1928, and his citations.

even those of rather closely related forms; and radially symmetrical ciliates apparently succeed in life as well as the extreme asymmetrical forms. It often appears as if the individual makes the best of the pattern it possesses.

If the morphological asymmetries of these forms are expressions or results of molecular orientations and aggregations, we still know nothing about the relation between a particular morphological asymmetry and a particular pattern of molecular character. It seems highly improbable, however, that molecular or supermolecular patterns develop independently of the activities and conditions in the metabolizing protoplasm in which they originate. In so far as they may be features of the morphological patterns of spermatozoa, protozoa, or other forms, they are evidently localized and integrated in an orderly manner in relation to some more general pattern, so that an individual with definite, species-specific asymmetries results. That the ordering and integrating factors must involve a spatial metabolic pattern seems beyond question, and the most general evidences of such pattern are the gradients. Moreover, experiment has shown so generally that these patterns do not arise autonomously in the cell or cell mass concerned but are induced by, or related in a definite manner to, parental pattern or to some factor in intra- or extraorganismic environment that it may at least be questioned whether organismic patterns ever do originate autonomously.

PATTERNS OF CERTAIN CELL WALLS AND SURFACES

Work with the ultramicroscope and the polarizing microscope and X-ray analysis have led to the conclusion that cellulose consists of molecular chains of variable length. A parallel orientation of these to form a definite pattern has been found in the cellulose layers of many plant cell walls and in plant fibers. Microscopically visible striae in cellulose layers of certain cell walls correspond closely with the pattern indicated by X-ray analysis and are regarded as resulting from aggregation of the parallel chains.

The cell wall of the alga *Valonia* is an interesting example. The *Valonia* thallus is primarily a single multinucleate, more or less spheroidal, or somewhat elongated cell, often attaining a length of several centimeters. A layer of protoplasm adjoins the wall, and inside this is the large vacuole. The cell wall consists of many cellulose layers, each of which shows microscopic striations in a certain direction. Polarization and X-ray studies of this wall indicate that each layer consists of cellulose chains in

parallel orientation, those of any one layer being inclined in direction to those of the layer adjoining on each side at an angle averaging somewhat less than a right angle. The chains of one set of layers constitute meridional systems with reference to the two poles of the cell; those of alternate sets, spiral systems centering at the same two poles. In short, the X-ray cellulose pattern shows a definite relation to a cell polarity of some sort.¹³ The *Valonia* cell exhibits physiological polarity: small uninucleate cells develop from the multinucleate protoplast in one polar region and form holdfasts or rhizoids, and cells giving rise to thallus buds form from the opposite polar region. This polar difference in behavior is similar to that in many plants and animals in which a polar gradient has been demonstrated; consequently, it is probable that a longitudinal gradient system is present in *Valonia*. At any rate, the cellulose pattern of the cell wall appears to be definitely related to, and probably determined by, a general protoplasmic pattern. Granting that such a relation exists, it does not account for the periodicity represented by successive layers or for the alternation of meridional and spiral orientations of cellulose chains constituting the layers.

In cell walls of multicellular plants, as far as examined, the cellulose micells or crystallites are, in general, parallel to the surface of the wall but may be parallel or transverse to the long axis of the plant or un-oriented. They are predominantly transverse in parenchyma cells of the *Avena* coleoptile, according to the optical evidence; but a change toward longitudinal orientation occurs when the cell wall is stretched longitudinally, though not when growth elongation by action of auxin takes place. In cell walls of the coleoptile epidermis the orientation is predominantly longitudinal and on longitudinal stretching becomes more completely so.¹⁴ Apparently cellulose orientations in these cells are determined by local intracellular conditions and relations to adjoining cells. Experimental work on effects of stretching, swelling, etc., on cellophane and plant fibers, as well as on cell walls, indicates that mechanical factors play an important part in orientation.

Evidence of orientation of particles is also found in chitinous cuticles and exoskeletons of various animals. An orientation of heteropolar molecules vertical to surfaces or phase boundaries is another effect of local factors in protoplasms. It does not appear, however, that the microscopic or ultramicroscopic patterns of cell walls or protoplasmic surfaces consti-

¹³ Preston and Astbury, 1937, and their citations of earlier literature.

¹⁴ See Bonner, 1935, and his citations; also, for general discussion, Seifriz, 1936, chap. xv.

tute the basis of spatial pattern of organisms. They are either patterns of dead products of metabolism or reactions of protoplasmic molecules to limiting surfaces, mechanical factors, and probably other factors. They may be definitely oriented with respect to an organismic pattern already present or a reaction to purely local conditions.

SYMMETRY IN CERTAIN MULTICELLULAR BUD PATTERNS

The terms "bud" and "budding" have often been used loosely by biologists to include forms of development representing fission rather than budding. As regards axiate organisms, budding may perhaps be defined and distinguished from fission as a localized activation in an organism, resulting in development of a new polar pattern of individual, organ system, or organ. In fissions of axiate forms the polar pattern is not an entirely new pattern but a reconstitution in relation to a part of the parental pattern. Buds apparently represent the beginnings of polar pattern. In other types of axiate agamic development and in embryonic development pattern is already present when development begins. In certain types of agamic development, usually called "budding"—for example, intercalary development of zooids in many annelids and the so-called "stolons" in certain Syllidae—the "bud" continues posteriorly the axiate pattern of the parent. In origin it is essentially similar to a reconstitution, but it is more or less physiologically isolated and may become a sexual individual, differing in form from the asexual parent. Whether we regard these zooids as products of budding or fission is perhaps a matter of opinion, for in origin they show resemblances to both.

The only pattern at present distinguishable in early stages of the simplest bud forms is a radial gradient pattern, representing the radial decrease of activation from a central region of primary or most intense activation. In consequence of differential growth, or probably in some cases differential cell migration, the radial gradient system becomes a longitudinal axial system (pp. 16-21). There is no evidence that any other pattern than this gradient system and its changes is necessary for axiate development and differentiation from a bud. However, even though the bud represents a new axiate pattern, certain features of its pattern may be related to, and determined by, the pattern of the parent individual. Lateral buds of many multiaxiate plants—for example, many conifers—give rise to branches dorsiventral and bilateral in pattern in consequence of their relation to the main axis. If the growing tips distal to such a branch are removed, it becomes erect and radial in its further

development. The dorsiventrality and symmetry or asymmetry of the leaf bud and of parts of flowers develop in a particular relation to the axiate pattern of the part on which they arise. The whole pattern of the multiaxiate plant represents a series of such relations.

In certain coelenterates somewhat similar relations appear. Various workers with hydra have observed that the pattern of tentacle appearance on the bud of *Pelmatohydra oligactis* is not primarily radial but is related to the longitudinal axis of the parent hydra in such a way that

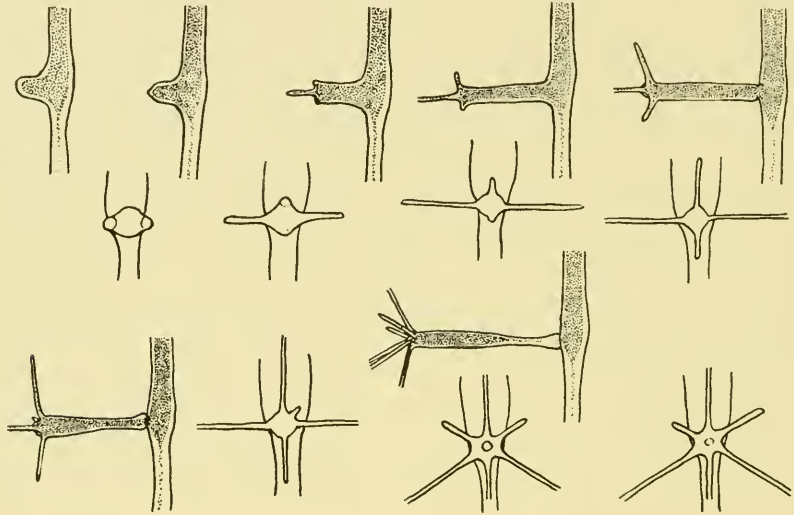


FIG. 203.—Order of tentacle appearance in bud of *Pelmatohydra oligactis*. Upper row, side views of stages up to four tentacles; second row, apical views of stages from two to four tentacles; lower row, lateral and apical views of later stages (from Rulon and Child, 1937a).

the bud is temporarily “dorsiventral.”¹⁵ The first two tentacles appear simultaneously on opposite sides of the bud in a plane at right angles to the polar axis of the parent; the third tentacle is midway between the first two on the side toward the apical end of the parent; the fourth opposite the third; and the fifth and sixth between the first two and the third, either simultaneously or one earlier than the other. Stages of this development are shown in Figure 203. Gradually the tentacles become equal in length and equidistant on the hypostome, and the dorsiventral pattern becomes radial. Although the bud represents a certain degree of physiological isolation and a new longitudinal axis at right angles to the old, the parental pattern still plays a part in determining the order of

¹⁵ Rulon and Child, 1937, and their citations.

tentacle development. An interpretation suggesting that parental dominance and gradient difference on the two sides of the bud are not entirely obliterated in early bud stages has been offered (Rulon and Child, 1937, p. 10). In some of the corals the apical zooid of the colonial axis is radial; the lateral zooids, originating as buds from its basal region, are dorsiventral and bilateral, with the median plane passing through the long axis of the colony or branch. Each of these lateral zooids, like the lateral branch of a multiaxiate plant, is apparently capable of becoming a radial apical zooid when sufficiently isolated physiologically or physically (Wood-Jones, 1912).

Bryozoan buds represent new longitudinal axes, but their dorsiventrality is determined in definite relation to pattern already present in the region of the parent from which they arise. Statoblasts of phylactolaematus bryozoa, often regarded as internal buds, develop from the funiculus, a strand extending from the end of the aboral prolongation of the stomach, the caecum, to the aboral wall of the zooecium. The funiculus is described as consisting externally of mesoderm and internally of ectoderm, which enters it from the wall of the zooecium in early stages. The statoblasts, more or less lentoid in form, develop successively from near the caecal end of the funiculus. An axiate pattern coincident with the short diameter and evidently determined at the time of their formation is present, and pattern of later development has a definite orientation with respect to it. The dorsiventrality of the polypid and localization of the primary budding region are apparently also predetermined in the statoblast but are not evident until its development. The relation of statoblast pattern to the pattern of the parent zooid is not entirely clear. In some forms—for example, *Cristatella*—the short diameter of the statoblast is said to be coincident with the long axis of the funiculus, and statoblast formation is a sort of strobilation of the funiculus. In other forms (*Plumatella*) the short diameter is apparently at right angles to the funicular axis, and the statoblast is perhaps to be regarded as a lateral bud. In either case it seems probable that the statoblast is primarily a bud with a new polar axis, like other buds, but that certain other features of its pattern are determined in relation to parental pattern.

Various types of budding appear in ascidians: some of the types of development commonly called budding resemble fission rather than budding, and certain others approach development from cell aggregates. The association of certain types of budding in many forms with a depression or degeneration of a parent individual suggests physiological or physical

isolation as a factor in the origin of buds. In the repetitive budding of pelagic tunicates the orderly periodicity is difficult to account for, except in terms of dominance and physiological isolations.

Apparently the longitudinal axis of the tunicate bud which originates as a local activation represents transformation of a more or less radial pattern into a longitudinal pattern by differential growth, as in other buds; but dorsiventrality and asymmetry in these buds are definitely oriented in relation to the parent pattern or to a local factor in the stolon or other budding region. The winter buds of some forms are more like fissions than buds.¹⁶ They, or some of them, apparently reconstitute from the part of the parent pattern persisting in them, but the reconstitution of experimentally isolated pieces of ascidian bodies and stolons indicates that new polarities may arise in such pieces in relation to section and perhaps to other factors (p. 369). But whether the specific asymmetry pattern of the ascidian can originate in relation to a new polarity and independently of parental pattern seems not to be known.

The sponge gemmule is sometimes regarded as a sort of internal bud but probably resembles more closely an aggregation of dissociated cells than a bud, except that it does not contain all the differentiated tissue cells that may be present in an aggregate. Like the aggregate, the cell mass of the gemmule apparently does not possess a definite organismic pattern until subjected to an environmental differential, usually that between free surface and surface in contact, presumably an oxygen differential. The canals center, and oscula develop on the free surface.

MULTICELLULAR AXIATE PLANT PATTERN FROM CELL AGGREGATES

In certain plants multicellular axiate patterns, often multiaxiate patterns, develop from aggregates of myxamoebae, of diatoms, or of hyphae of fungi. These patterns are orderly and definite in character, and some of them attain considerable morphological differentiation. The features and problems they present are, or should be, of much interest to the developmental physiologist.

DEVELOPMENT OF PATTERN IN ACRASIEAE

In this small group of plants, often regarded as more or less closely related to the myxomycetes, axiate pattern originates in definitely directed motor reactions of separate amoeboid cells. Two forms, *Polyspondylium*

¹⁶ See, e.g., Berrill, 1935.

and *Dictyostelium*, have received considerable attention.¹⁷ The following account is based on the recent papers of Harper and Arndt. Cultured in dung decoctions with agar or agar-bouillon and *Bacillus coli*, the spores give rise to small amoeboid cells (myxamoebae); these move about indefinitely, feed and divide for a time, but sooner or later begin to move in more or less definite streams, at first in all possible directions, constituting a network of moving amoebae. According to Arndt, appearance of directed streaming of the amoebae is associated with lack of nutrition in the culture. As the streaming continues, centers of aggregation appear, apparently entirely by chance, except that they are more likely to form in dryer parts of the culture—for example, at the margins (Harper). Harper and Arndt agree, however, that the amoebae react primarily to a condition produced by other amoebae rather than to local differences in substrate otherwise produced. Streaming of amoebae in the vicinity of an aggregation becomes directed toward it; and as the aggregation increases in size, the streaming becomes increasingly definite and the area of centered streaming greater. Evidently the differential to which the amoebae react represents a radial gradient system of variable scale, presumably due to diffusion of a substance produced by the amoebae. Early-stage aggregations may be only temporary and may disappear completely by alteration in direction of reaction of the amoebae, or an aggregation may change its position (Arndt). But once an aggregation is well established, the amoebae continue to stream toward it and build it up above the surface of the nutritive substrate, forming the beginning of an axis. The further development of *Polyspondylium*, as described by Harper, is of great interest. Figure 204, A, is an approximate representation of the chief paths of streaming of amoebae toward a center, as shown in a photomicrograph by Harper (1929). The developing axis, rising above the substrate, is indicated at *a* in the figure. The amoebae continue to arrive at the center of aggregation, move up the axis in or on the slime secreted by those already there, and come to rest at the top, forming an elongated cylindrical structure, the sorogen. From the basal part of the aggregation a slender stipe forms by transformation (differentiation?) of amoebae into cells resembling plant parenchyma with complete cessation of amoeboid activity. The stipe elongates by continued transformation of amoebae into stipe cells at its distal end. The resulting form is an

¹⁷ Brefeld, 1869, 1884; van Tieghem, 1880; Olive, 1902; Potts, 1902; von Schuckmann, 1925; R. A. Harper, 1926, 1929, 1932; Arndt, 1937.

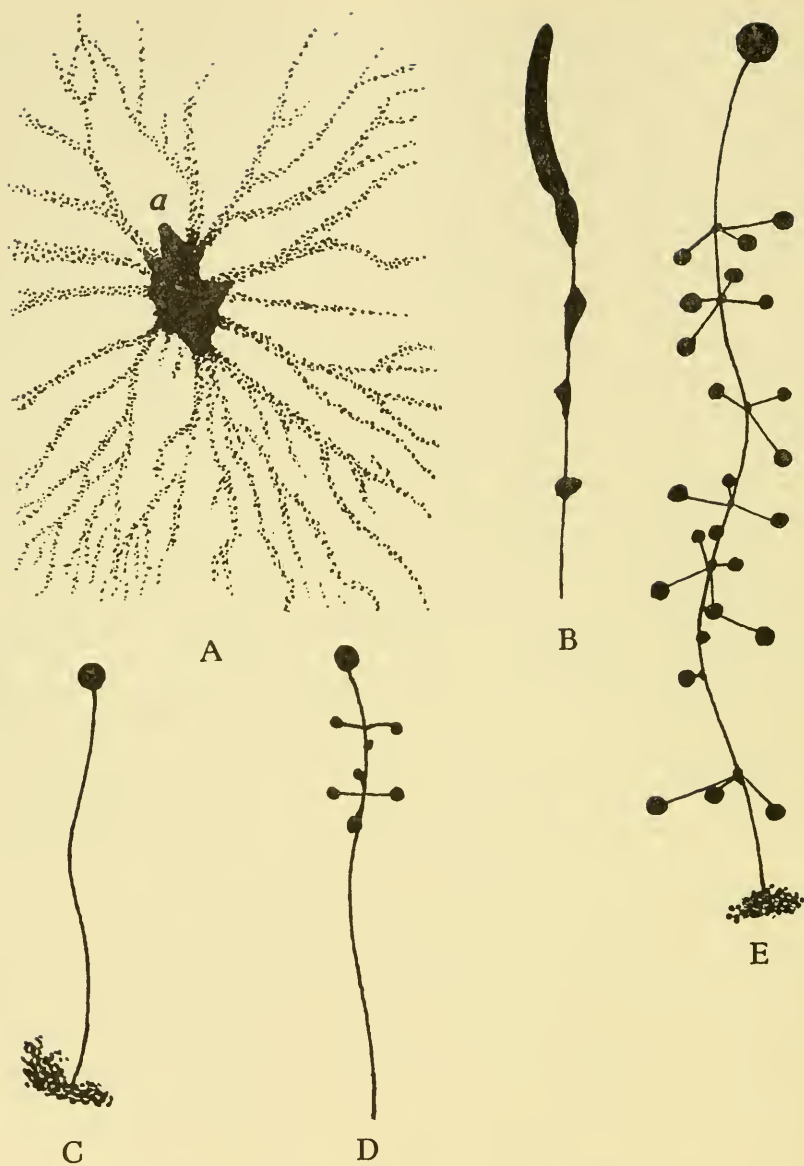


FIG. 204, A-E.—Development of axiate pattern in *Polyspondylium*. A, directed streaming and aggregation of myxamoebae and beginning of axiate development at *a*; B, origin of branches from masses of amoebae successively constricted from basal region of sorogen and “left behind” as elongation continues; C, fully developed, unbranched individual; D, E, branched individuals (after R. A. Harper, 1929).

elongated cylindrical mass of amoebae borne on a slender stipe. This differentiation into stipe and sorogen perhaps results from arrival of amoebae at the summit of the aggregate more rapidly than they are transformed into stipe cells, so that conditions in the mass at the summit become different from those in the developing stipe and perhaps retard the transformation.

Branching in *Polyspondylium* occurs as follows: At a certain stage a constriction appears near the base of the cylindrical sorogen, and a mass of amoebae is "left behind" on the stipe as it elongates further. In Figure 204, *B*, three such masses have been isolated, and a fourth is constricting off at the base of the sorogen. Each of these masses on the stipe gives rise to branches, usually a whorl. The branches develop in much the same way as the main axis but at an angle to it; that is, the amoebae now react plagiotropically. In Figure 204, *C*, *D*, and *E* show fully developed individuals—unbranched (*C*), with two whorls (*D*), and with seven whorls (*E*). Development of *Dictyostelium* is, in general, similar except that it usually does not branch. In these forms cell division is apparently limited to the earlier stage of feeding and undirected movement of separate amoeboid cells and has not been observed during morphogenesis.

At no stage in this development do the amoeboid cells form a plasmodium, as in myxomycetes. They stream and aggregate as separate cells, and even the cylindrical sorogen consists of cells capable of resuming amoeboid movement as separate cells, if again brought into contact with the culture substrate, and of giving rise to a new sorogen.

As regards interpretation of the origin of this axiate pattern, Harper holds that there is no reason "for assuming any superchemical or physical stimuli or the action of organizing or regulating principles associated with the multicellular organismal condition resulting from the aggregation of the myxamoebae. The behavior of the pseudoplasmodium as a whole is the sum of the behaviors and reactions of the individual myxamoebae" (Harper, 1929, p. 237). Arndt does not go beyond the assertion that the culture of myxamoebae is a harmonious-equipotential system.

While no data are at hand concerning the presence or absence of a gradient in the developing axis, there is every reason to believe that a gradient is present. Amoebae are being continuously added at the free end; and when they arrive there, they are obviously in a different condition from earlier arrivals which have lost motility and have come to resemble ordinary plant cells. The stipe develops progressively as the amoe-

bae continue to arrive and undergo change in condition. Presumably oxygen tension is considerably lower in the interior or more basal parts of the aggregate than at the free end. That this axis in its earlier stages is a simple gradient in physiological condition of the constituent amoebae and the cells developing from them appears highly probable. The change from aqueous to aerial environment, as the aggregation rises above the substrate, may also be a factor in bringing about the change in condition in the cells; and its effect is probably, within limits, a function of time of exposure. If this is the case, a longitudinal gradient in physiological condition must result with the high end apical, where the active amoebae are arriving. Moreover, the aggregation itself may determine an environment which alters the physiological condition of the amoebae; and any such change probably progresses with time, so that older parts of the aggregate have undergone more change than younger, and a gradient results. In fact, it seems improbable that an aggregation such as occurs in these forms is possible without development of a gradient pattern, and the morphogenesis suggests such a pattern.

The successive separations by constriction of masses of amoebae which give rise to branches suggests a dominance with limited range at the stages concerned and successive physiological isolations, as continued arrivals of amoebae in the apical region elongate the sorogen. The physiologically isolated mass apparently undergoes some activation, as in other organisms, forms local aggregations, and these develop as branches. Harper's photomicrographs of the branch-forming masses suggest a slight local dominance at the nodes; that is, the forms of the masses (Fig. 204, *B*) and in many cases differences in size of sori and lengths of stipes in a single whorl (Fig. 204, *D*, *E*) suggest that the branches of a whorl do not develop simultaneously but in sequence.

This development differs in certain respects, though apparently not fundamentally, from development of axiate pattern in cell aggregates of sponges and hydroids (pp. 348, 418). Here the aggregation and the axiate pattern result from a directed motor reaction and probably from differential exposure to certain environmental factors. In sponge and hydroid aggregates aggregation supposedly results from chance contacts of cells, and axiation is determined by a spatial environmental differential. That either the myxamoebae or the dissociated sponge or hydroid cells possess any inherent characteristics that enable them to originate an orderly and definite multicellular axiate pattern independently of a chronological or spatial differential in environmental factors is neither probable nor indi-

cated by the data at hand. The early aggregate, such as in Figure 204, *A*, is apparently not essentially different in pattern from an adventitious plant bud, but its further development depends on addition rather than on growth and division of cells.

THE PSEUDOTHALLI OF OTHER FORMS

Certain species of diatoms give rise to axiate systems resembling the thalli of algae but consisting of diatoms, apparently not even in contact but imbedded in a firm jelly-like substrate secreted by them. The growth form of one of these diatomaceous pseudothalli is shown in Figure 205. The branches are flat and thin in the plane of the paper. Different branches of a single pseudothallus are approximately equal in width; and,

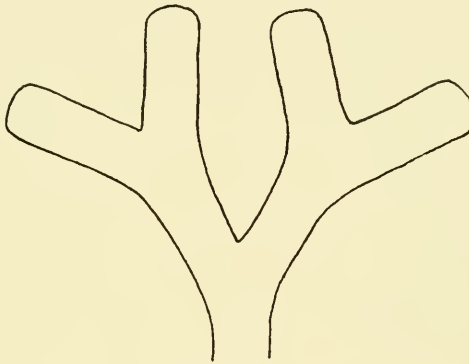


FIG. 205.—Part of a diatomaceous pseudothallus

as it attains a certain length, each branch undergoes dichotomous division. This pseudothallus consists of diatoms, more or less regularly arranged in longitudinal rows in the jelly-like secretion, their long axes coinciding in direction with the rows and the axiate pattern. This definite, orderly axiate pattern must result from definite, orderly relations between the diatoms giving rise to it. If all grew and divided at the same rate, such a form could not arise. Evidently the chief, or only, region of growth and division is apical; but the individual diatoms of the apical region must be integrated in some way into a system resembling, in certain respects, the growing tips of various algae, in which very similar thallus patterns appear. Differential susceptibility and differential reduction indicate a gradient in these pseudothalli with high end apical, and extending for at least a short distance from the apical ends of the branches (Child, 1919*f*). Some other diatoms give rise to branching systems of more or

less orderly character in which the main axis and branches consist of the secretion and the diatoms are in groups at the tips. Here, also, some degree of integration and apparently some degree of dominance are involved as regards growth and division of the different groups; but these forms are less regular in growth than the pseudothalli, and nothing is known concerning possible physiological differences in the diatom groups of different branches. Obviously, the patterns of these supercellular integrations cannot be inherent in the individual diatoms but must originate in reactions to physiological conditions determined by cells as environment of other cells and also to the external environment.

DEVELOPMENT OF MULTICELLULAR AXIATE PATTERN IN CERTAIN FUNGI

In the mushrooms and toadstools among the basidiomycetes and in some forms among the ascomycetes—the morel and related species—the vegetative stage consists of indefinitely branching hyphae, each consisting of a single series of cells; these hyphae usually form a subterranean feltwork, the mycelium. Each hypha and branch represents an axis with growing tip, and a gradient has been demonstrated in young, growing hyphae of certain fungi; but hyphae of basidiomycetes have not been examined. Development of the fruiting body or sporophore, the mushroom, toadstool, or morel, begins as an aggregation of hyphae at some region of the mycelium. By more or less parallel upward growth of hyphae the aggregation rises above ground as a small rounded structure. This elongates by further, more or less definitely directed growth; and the form of stipe and pileus or cap, inclosed in a loose feltwork of hyphae, the volva, becomes distinguishable in it. In forming the pileus the hyphae change direction of growth; and in development of “gills” or other parts from which the basidia (modified ends of hyphae) arise and form spores, further changes in direction of growth of hyphae in orderly and definite relation to pattern of the whole are involved.¹⁸ In some of the basidiomycetes there is considerable further regional differentiation, and pigmentation of the upper surface of the pileus is characteristic of some forms. Even after development of pileus and stipe, every cell of *Coprinus* is capable, on return to nutritive medium, of forming vegetative mycelium, and from this, new sporophores. In certain forms evidence of dominance and physiological isolation appears in the dependence of branching on inhibition of the apical region (Brefeld).

The origin of axiate pattern in the mushroom or toadstool differs from

¹⁸ See, e.g., Brefeld, 1876; Magnus, 1906; P. Köhler, 1907.

that in *Polyspondylium* in that pattern results from directed growth of individual axes instead of directed movement and aggregation of individual cells, but in both cases pattern originates in the common reaction of individual cells to some factor or factors in their environment.

CONCLUSION

It should be evident from this chapter that the problems of pattern in development are not limited either to embryonic development or to fissions, buds, and reconstitutions. While there are various types of development about which we know little or nothing, the evidence from observation points, in general, in the same direction as the experimental evidence in indicating that in some, if not in all of these, developmental pattern is a reaction of a specific protoplasm to factors in its environment within a parent organism or external to it and that pattern is primarily dynamic in character rather than structural. Moreover, development of a definite and orderly axiate pattern from directed motor reactions and aggregation of primarily separate, amoeboid cells, from a multitude of diatoms in a jelly-like secretion, and by directed growth reactions of fungus hyphae suggests, like the experimental evidence from cell aggregates, buds, and reconstitutions, that axiate organismic pattern is, or may be, something different from the primary pattern of the cell or of a protoplasm, often on a larger scale, and superimposed from without. But whatever the initiating factor or factors, the character of development within the pattern depends on the specific constitution of the protoplasm concerned. Axiate pattern in the aggregates of *Polyspondylium* myxamoebae develops as *Polyspondylium*; in an aggregate of *Corymorpha* cells, as *Corymorpha*; and in a spermatid of a particular species, as a spermatozoon of that species.

CHAPTER XVI

ORIGIN AND NATURE OF EMBRYONIC PATTERNS THE PROBLEMS AND THE EVIDENCE

QUESTIONS of the origin and nature of pattern or "organization" of the animal egg have been the subject of discussion and speculation since the beginnings of embryological investigation. This has been due in part to the fact that the ovarian oöcyte is inaccessible to most present experimental procedure but perhaps also in some measure to the very general belief that embryonic development is of primary significance and that all other types of development have only a secondary interest. If we attempt to interpret embryonic pattern and development in terms of what experiment has shown concerning the simpler forms of development, we may perhaps at least come nearer agreement concerning certain questions of embryonic pattern.

Preceding chapters have dealt chiefly with experimental evidence concerning the characteristics of the spatial or regional pattern of individual development, and the question of the origin of pattern under natural conditions has received little attention. The earliest distinguishable pattern appears to consist of regional differences of some sort on a molar or morphological, rather than a molecular or micellar, scale. Protoplasms are complex colloidal and crystalloidal physicochemical systems, and organisms appear to be systems of quantitatively or qualitatively different protoplasms. The series of changes constituting what we call "living" take place in many kinds of protoplasms, but an organism is an integration in an orderly and definite pattern of different rates or kinds of living. The question of the origin and nature of this pattern is of fundamental importance for our conception of the organism and of development.

If developmental pattern is primarily independent of environment, either it must be continuously present as a spatial pattern through all the cell divisions from one generation to another, or it must originate autonomously in the cell or cell mass concerned. The assumption of an "intimate structure" of some sort, adequate, *ex hypothesi*, as a basis for pattern, is only a statement of the problem in terms of structure. The Roux-Weismann theory of qualitative nuclear division states the prob-

lem in terms of distribution of hypothetical hereditary units but tells us nothing about the ordering and controlling principle determining the distribution so that an orderly organism of definite character results. It would seem that this principle must be endowed with superhuman intelligence and with knowledge of the end to be attained in order to get each determinant into its proper cell and to supply the cell with accessory determinants to provide for possible regeneration. The theory is implicitly teleological.

Driesch, assuming that developmental pattern is independent of environment in origin, drew the logical conclusion that a metaphysical ordering and controlling principle, the *entelechy*, is necessary. Various other authors have assumed an inherent, form-determining principle, teleological in character. As a matter of fact, the conception of developmental pattern as independent of environment seems to demand a teleological principle. If we reject the hypothesis of a metaphysical origin of organismic order and pattern, the question whether autonomy of pattern is possible on any other basis arises. Can the gene system alone originate a spatial pattern in a cell or a cell mass? Or is the intimate structure of molecular or other character, so often postulated as the basis of pattern, an inherent property of protoplasts? If not, can it arise spontaneously, that is, without relation to any external factor?

It was held by Rabl (1885) and others that the nucleus possesses polarity coincident with the axis of the mitotic spindle, since the recurved middle portions of the chromosomes lie toward the spindle pole. However, even if cell polarity is of this sort, it does not account for polarity of a multicellular organism, for polarities of the two cells resulting from division are in opposite directions. A nuclear polarity indicated by aggregation of chromatin toward a certain region of the nuclear periphery appears frequently under certain conditions or at certain stages but is obviously a reaction to something outside the nucleus rather than a primary factor of pattern. The general nuclear pattern and the chromosome patterns are apparently entirely different from the pattern of the organism. How a gene, a group of genes, or the whole gene system can originate autonomously in the cytoplasm, a spatial pattern involving polarity and symmetry or asymmetry, is not evident. Each nucleus supposedly contains the whole gene system. "Each cell inherits the whole germ plasm" (Morgan, 1919, p. 241). If different gene effects occur in different cells or in different localized regions of the same cell, it seems that they must be localized in relation to something, a differential or pattern of some sort

independent of particular genes and of the nuclear or chromosomal pattern. A discussion by Morgan (1919, pp. 241-46) under the heading, "The Organism as a Whole or the Collective Action of the Genes," seems to imply that organismic pattern is entirely a matter of gene action but does not show how this is possible. In an earlier discussion a very different view was advanced, that differentiation depends on reaction between hereditary factors and regional differences in the egg and embryo which are independent of them.¹

With progress of experimental analysis it has become increasingly difficult to separate organism and environment. Not only functional patterns of mature individuals but developmental patterns are highly susceptible to environmental conditions, and it is impossible to conceive an organism except in relation to environment. If life is "the continuous adjustment of internal relations to external relations," as Spencer put it, or a continuous equilibration in reaction to environment, then life is the behavior of protoplasmic systems, and developmental pattern represents certain aspects of that behavior (Child, 1924*b*). Surface-interior pattern is so obviously a reaction to environmental factors that it need not concern us further here. In addition to surface-interior pattern, all but the simplest organisms possess axiate pattern, and evolution is very largely a matter of modifications of this pattern and of the reactions within it.

The view commonly held at present is that, whatever the nature of embryonic pattern, it does not originate autonomously in the egg but originates in relation to its intraorganismic environment, that is, to factors in the relations of the oöcyte to the parent organism.² According to Goldschmidt, however, these factors are controlled by the gene system of the parent. He regards the cytoplasmic pattern as a stratification, the different levels providing substrata for activation of different genes. However, cytoplasmic stratification involves quantitative or qualitative regional metabolic differences; and these, rather than the stratification itself, are the effective factors in development. On this basis heredity alone does not provide a pattern for development but represents the potentialities of the species- or individual-protoplasm; for the realization of any of these potentialities in development certain environmental conditions are necessary. The course of embryonic development is altered when essential factors of the environment are altered; the potentialities realized under ex-

¹ This view appears unaltered in a revised edition of the book by Morgan, Sturtevant, *et al.*, 1923 (first published in 1915).

² See, e.g., E. B. Wilson, 1925, pp. 1021-25; Goldschmidt, 1938, pp. 200-262.

perimental conditions may give a pattern very different from that which we call "normal."

SOME EMBRYONIC PLANT PATTERNS

Botanists have apparently been less generally concerned than zoölogists with questions of the origin and nature of embryonic pattern, but botanical literature contains many interesting and suggestive data and raises various questions concerning pattern. A few of these are briefly noted.

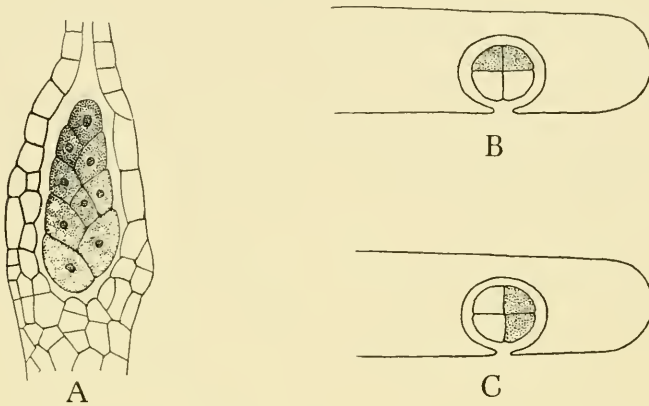


FIG. 206, A-C.—Embryonic pattern in moss and fern. *A*, embryo of a moss with part of archegonium in longitudinal section (from Child, 1915*c*, after a preparation loaned by W. J. G. Land); *B*, *C*, diagrammatic, indicating relations of embryonic regions in ferns to archegonium and gametophyte, anterior end of prothallium to the right (after Coulter, Barnes, and Cowles *Textbook of Botany*, 1910).

CRYPTOGAMS

The case of the alga *Fucus* and related forms has been discussed in another connection (p. 423). Among the bryophytes the polarity of the embryo is generally coincident with the longitudinal archegonial axis, the apical end being directed toward the neck of the archegonium. Figure 206, *A*, the embryo of a moss in longitudinal section, shows this axial relation and indicates the presence of an apicobasal gradient in physiological condition by the basipetal decrease in depth of staining and increase in size and vacuolation of cells.

Axial relations of the embryo to the archegonium and to the gametophyte differ in different groups of the pteridophytes, suggesting that in some the archegonium may be the factor determining the primary pattern,

in others the axiate pattern, of the gametophyte. The gametophytes of most ferns are flattened, axiate prothallia with an apical growing cell or cell group, with dorsiventral differentiation, and with archegonia developing on the ventral side.³ The first division of the zygote is in a plane transverse to the axis of the archegonium in most forms, but in Polypodiaceae it is in a plane passing through the axis of the archegonium and transverse to the long axis of the prothallium, that is, in definite relation to its environment. When it is transverse to the archegonial axis, the second division plane is at right angles to it; when it passes through the archegonial axis, the second plane also passes through that axis at right angles to the first, and the third is transverse. The four quadrants or four pairs of octants resulting from these divisions are said to represent more or less exactly four regions of the developing plant—cotyledon, stem, root, and foot, the latter a temporary nutritive organ connecting the embryo with the gametophyte. The fern embryo is then temporarily a bilateral form, but the relation of the four regions to archegonial and gametophyte pattern differs in different groups. For example, the diagrammatic Figure 206, *B*, indicates the relation believed to exist in certain forms (order Marattiales), the foot developing from the two unshaded quadrants next to the neck of the archegonium, stem, cotyledon, and root, from the two shaded quadrants. The relations in the Polypodiaceae are indicated in Figure 206, *C*, a lateral view of the eight-cell stage. The two dorsal anterior cells (upper shaded quadrant of the figure) give rise to stem; the ventral anterior pair, to cotyledon; the dorsal posterior pair, to foot; the ventral posterior pair, to root. In Figure 206, *B*, the embryonic pattern is apparently related to the archegonial axis, perhaps secondarily to the longitudinal axis of the gametophyte; in Figure 206, *C*, the relation is somewhat different, but since it is apparently constant it is significant. In *Equisetum* the first division is transverse to the archegonial axis, the cell next to the base forming the foot or the foot and root, the other cell, stem and cotyledon.

In *Lycopodium* and *Selaginella* the first division is transverse to the archegonial axis; and the cell next to the neck, or a descendant of it, becomes the suspensor, an embryonic organ not present in other pteridophyte groups; and cotyledon and stem develop from cells next to the base of the archegonium; but in later development the embryonic axes undergo change in direction, apparently in relation to the gametophyte

³ Archegonia are on the dorsal side of the prothallia in Ophioglossales, and in *Marsilea* the single archegonium is apical on the apparently radial gametophyte.

pattern. The development of *Selaginella* affords an interesting example of these changes. The megaspore has an apicobasal axis; and development of the apparently radially symmetrical megagametophyte is at first limited to the apical region, the basal region which contains much starch becoming cellular only later. The apical region of the gametophyte in which archegonia develop is exposed by rupture of the spore wall over it, but most of the gametophyte remains within the spore wall (Fig. 207, *A*). Archegonial and gametophyte axes are in the same direction. The first division of the zygote in *S. martensii* is transverse to these axes, as indicated in the two-cell stage in Figure 207, *A* (Bruchmann, 1909). From the cell next to the neck of the archegonium the suspensor develops and elongates, pushing the embryo deep into the gametophyte tissue; the other cell gives rise to the embryo. This embryonic cell divides vertically, and each resulting cell again vertically. Two of the embryonic cells are shown in Figure 207, *B*. From one of the four cells resulting from the first two divisions the apical cell of the stem is separated by a diagonal wall (*a* of Fig. 207, *C*). As development progresses, the embryonic axis gradually bends to one side (Fig. 207, *D*) and finally more or less toward the apical region of the gametophyte, the foot develops toward the base of the gametophyte, and the rhizophore opposite the stem (Fig. 207, *E*). At this stage the embryo with suspensor and foot is a bilaterally symmetrical individual, but how change in direction of the embryonic axis which results in definition of a median plane is determined seems not to be known. The gametophyte is apparently radial; and, according to Bruchmann, gravity is not concerned. Conceivably the change in form from that of Figure 207, *C*, to that of Figure 207, *E*, may result from a reaction of the stem axis to an apicobasal differential in the gametophyte, or it may be associated with development of the large foot. But whatever the factors concerned in these changes in form and direction of growth, it seems evident that the pattern of early development shows a definite and constant relation to archegonial or gametophyte pattern or to both.

The gametophyte of *Isoetes* is somewhat similar to that of *Selaginella*, but relations of embryonic regions are apparently different. The first division of the zygote is transverse or inclined to the archegonial axis, the cell toward the base becoming foot, the other embryo, suspensor being absent. According to this account, the embryonic axis is opposite in direction in relation to its gametophytic environment to that of *Selaginella*. Perhaps early determination of the stem axis in *Selaginella* and apparently much later determination in *Isoetes* are concerned in this

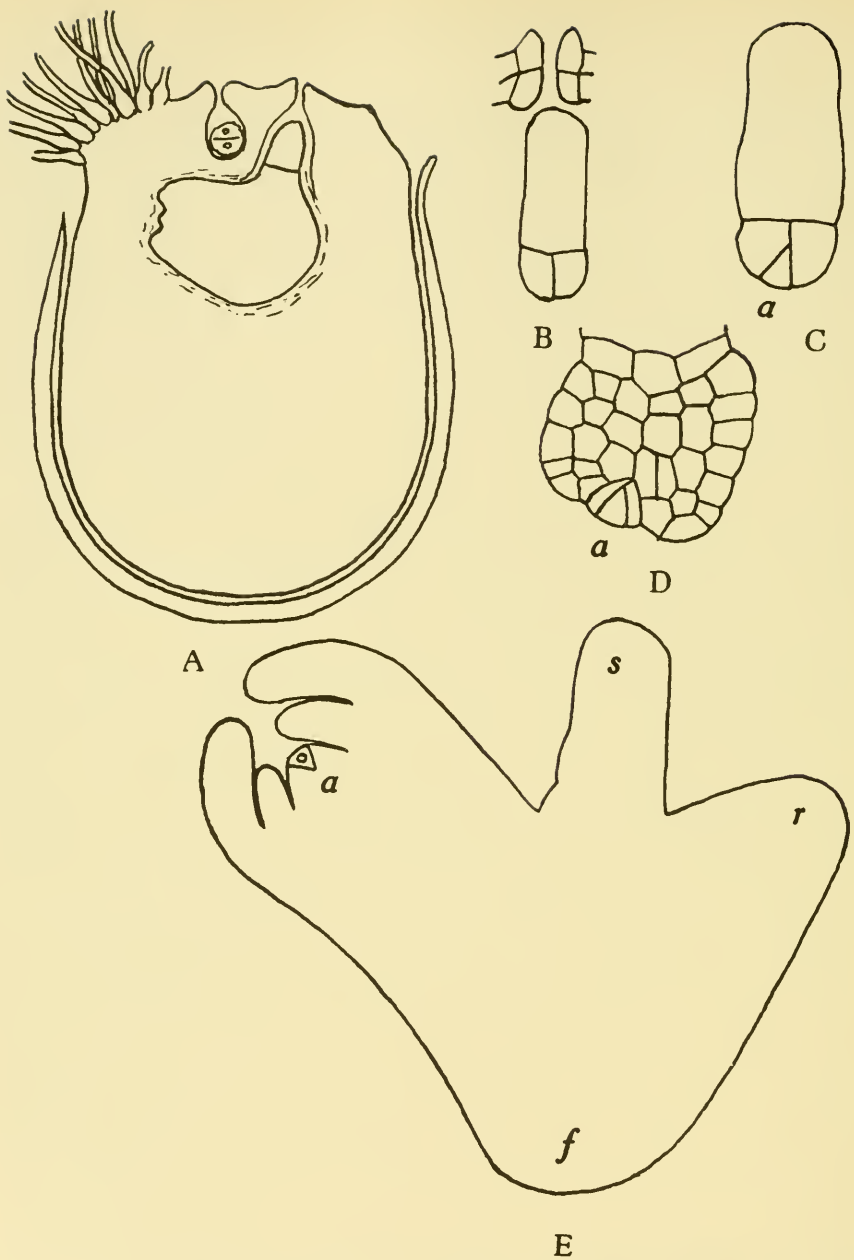


FIG. 207, A-E.—Stages in embryonic development of *Selaginella martensii*; all figures similarly oriented with respect to archegonium and gametophyte. A, outline of gametophyte with advanced embryo and two-cell stage and a group of rhizoids at upper left, spore coat still surrounding most of gametophyte; B, early embryo, suspensor and cells of archegonial neck; C, later embryo, showing apical cell, *a*, of stem; D, still later stage in longitudinal section; E, outline of advanced stage in median section; *s*, suspensor; *r*, rhizophore; *f*, foot; *a*, apical cell of stem (after Bruchmann, 1909).

difference. The parts of the *Isoetes* embryo are apparently determined in relations not greatly different from those attained in *Selaginella* at the stage of Figure 207, *E*.⁴

SPERMATOPHYTES

The gametophyte of the seed plants does not become an independent individual but is represented by cells of the embryo sac developing from the megaspore within the ovule. Its axiate pattern coincides in direction with that of the ovule; and the ovule originates much like a bud but becomes surrounded by one or more integumentary layers with an opening, the micropyle, at the free end. Earlier stages of gametophyte development usually consist of nuclear division without formation of cell walls in the cytoplasm of the megaspore within the developing ovule, cell walls, in so far as they occur, appearing later.

Gametophytes of gymnosperms (cycads, conifers, etc.) consist finally of many cells, and in most members of the group the eggs form within archegonia; but there appears to be a progressively earlier individuation of the egg in the evolution of the group, and in the most highly developed forms archegonia do not appear. When archegonia are present, they are usually at the micropylar end of the gametophyte with axes parallel to the gametophyte axis, but in certain gymnosperms they appear elsewhere. It has been suggested that their position is determined in reaction to the position of the entering pollen tube.⁵ In embryonic development the earlier divisions of the zygote give rise to a proembryo. The "basal" region of the proembryo, that is, the part toward the micropylar end of the ovule or the neck of the archegonium, becomes the suspensor; and in some forms other nonembryonic cells develop from it. The embryo develops from the "apical" region of the proembryo and stem tip, and cotyledons from the apical region of the embryo. The suspensor usually elongates, in some forms enormously (Fig. 208, *A*), and carries the embryo into the nutritive endosperm of the gametophyte. But in *Ginkgo* it is massive and many-celled; only the small-celled polar region is concerned in embryo formation; and stem tip and cotyledons develop later from its apex (Fig. 208, *B*). In the embryo of *Pinus* (Fig. 208, *A*) only the small cells at the tip of the suspensor form the embryo, and stem tip and cotyledons develop from its apical region.⁶ An apicobasal gradient, indicated by dif-

⁴ For more complete data on embryonic development of bryophytes and pteridophytes see G. M. Smith, 1938; Goebel, 1930; Campbell, 1918; Coulter, Barnes, and Cowles, 1910.

⁵ Coulter and Chamberlain, 1910, pp. 420-21.

⁶ In Fig. 208 and the following figures relating to spermatophyte development orientation is uniformly with embryonic pole upward.

ference in cell size, rate of division, vacuolation, staining properties (Fig. 208, *B*), or some of these differentials, very commonly appears in the proembryo or later, often developing gradually. Although the physiological factors concerned in determination of these axiate patterns in gymnosperms are not known, the patterns constitute a definite, orderly series, each new pattern originating in definite relation to a pre-existing pattern. That this relation indicates a physiological relation can scarcely be doubted.

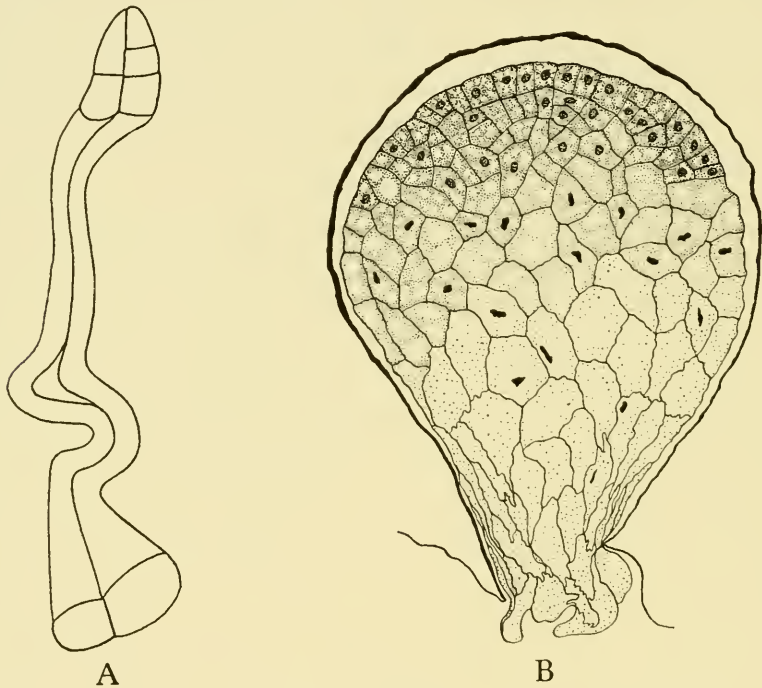


FIG. 208, *A, B*.—Embryonic stages of gymnosperms. *A*, greatly elongated suspensor cells of *Pinus* with embryo at tip (after Coulter and Chamberlain, *Morphology of Gymnosperms* [1910]); *B*, proembryo of *Ginkgo* (from Child, 1915*c*, reproduced from H. L. Lyon, 1904).

Development of the typical megagametophyte of angiosperms is diagrammatically outlined in Figure 209. Following the first nuclear division of the megaspore, the two nuclei come to lie near the two ends of the young embryo sac (Fig. 209, *A*); each of these divides again (*B*), and each of the four once more, giving four nuclei at each end of the sac (*C*). One nucleus from each group migrates toward the middle of the sac; the two usually fuse sooner or later, forming the endosperm nucleus. More or less definite cell boundaries develop about the three nuclei at the micropylar

pole, the three resulting cells being the egg and a synergid on each side of it (Fig. 209, *D*). The fate of the nuclei at the opposite antipodal pole varies in different families and species. They, or even the primary antipodal nucleus (Fig. 209, *A*), may degenerate; or more or less definite cell walls may develop about them, and these cells may become very large and undergo differentiation as nutritive organs or increase in number. Axiate pattern of the embryo sac is primarily coincident in direction with that of the ovule.

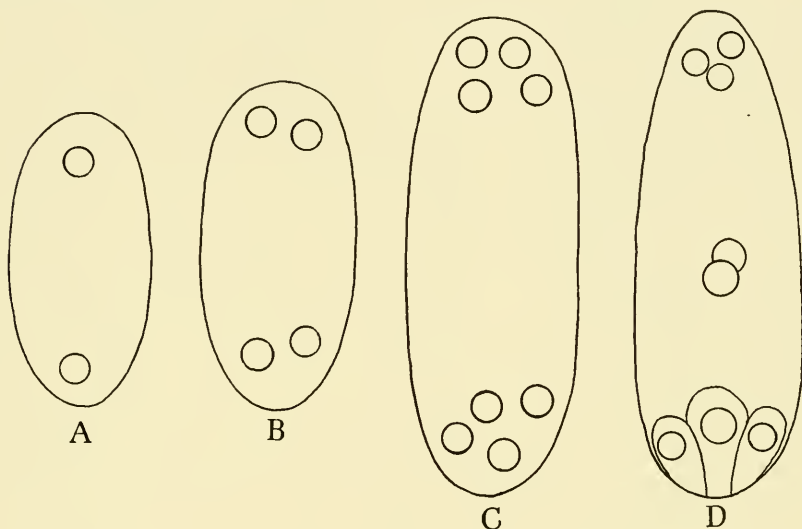


FIG. 209, *A-D*.—Diagrammatic outline of angiosperm gametophyte development. *A-C*, nuclei of first, second, and third divisions; *D*, egg between two synergids at lower pole in figure, antipodal nuclei at upper pole and members of primary endosperm nucleus in middle.

In development of the zygote the embryo is formed from the region of the egg which protrudes into the embryo sac, the “free” pole (upward in Fig. 209, *D*), the suspensor from the region toward the wall of the sac. Cotyledons and stem tip of dicotyledonous angiosperms develop from the “free” pole of the embryo. Figure 210 shows the axiate pattern of a dicotyledonous embryo and suspensor. The eight-cell stage of the embryo, of which four cells are shown in Figure 210, *B*, consists of four terminal, or apical, and four basal cells. From the four apical cells cotyledons and stem tip develop. The plane in which the two cotyledons develop is apparently also determined by factors external to the embryo.

The primary axis of the monocotyledonous embryos is oriented like

that of dicotyledonous forms (Fig. 211, A-C); but in most members of this group the single cotyledon develops from the terminal region, and the stem tip from cells lying laterally (s of Fig. 211, C). Presumably the side of the embryo on which the stem tip develops is determined by some factor in the embryonic environment, but thus far no consideration of this point has been found in the botanical literature consulted.

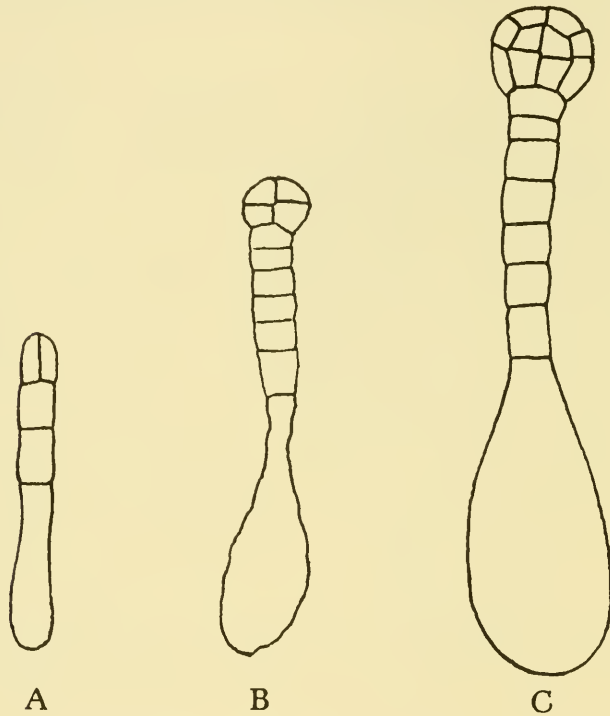


FIG. 210, A-C.—Three stages in early development of embryo and suspensor of a dicotyledonous angiosperm, *Capsella bursa-pastoris*. A, two-cell stage of embryo; B, one side of eight-cell stage; C, later stage (after Coulter and Chamberlain, *Morphology of Angiosperms*, 1903).

Polyembryony occurs in many spermatophytes with development of embryos, not only from single early cells of the primary embryo but from other cells of the gametophyte and even from cells of the nucellus, which is sporophyte tissue surrounding the gametophyte. In various gymnosperms polyembryony is normal or usual. In the pine, for example, each of the first four embryonic cells, products of two vertical divisions, has a suspensor cell, and each may form an embryo; or a single embryo may develop from all four. In other conifers also, single cells of early embry-

onic stages may give rise to whole embryos, some degree of dissociation being apparently the initiating factor. The proembryo of *Ephedra* forms by nuclear division without cell formation up to eight nuclei, after which cell formation occurs, and each of the eight cells gives rise to an embryo and suspensor.

Among angiosperms numerous cases of polyembryony have been recorded. In some there is multiplication of embryos by budding from the

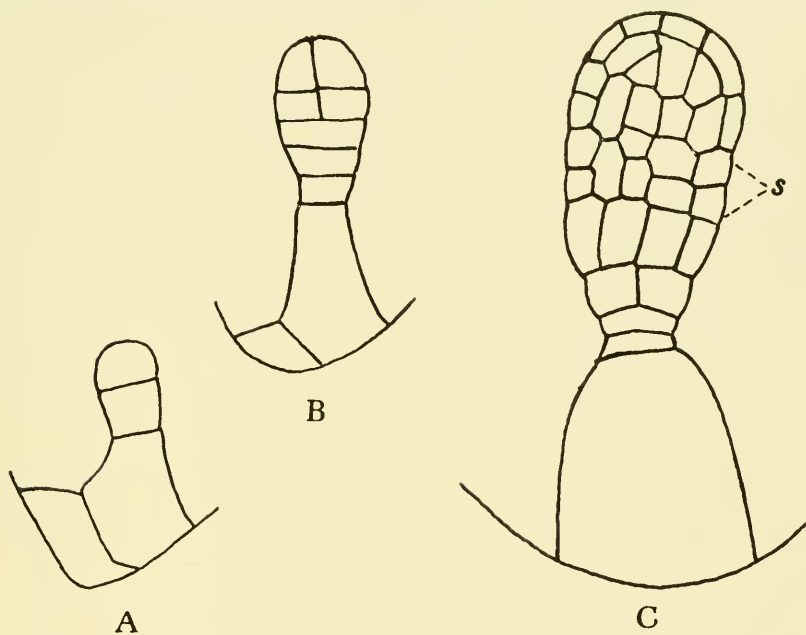


FIG. 211, A-C.—Three stages of embryo and suspensor of a monocotyledonous angiosperm, *Sagittaria variabilis*. A, B, early stages with primary axis oriented as in dicotyledonous forms, one synergid below embryo at left; C, later stage, the single cotyledon from the terminal region, the stem tip from the lateral cells, *s* (after Schaffner, 1897).

suspensor, but usually only one persists. In these cases all embryos develop from cells of the zygote. In others a synergid may be fertilized and give rise to an embryo or may develop without fertilization. Embryos may develop from antipodal cells and apparently also from an endosperm cell. They may also originate as buds from nucellar tissue outside the embryo sac, more commonly from the region about the micropyle than elsewhere. These cases of polyembryony present several points of interest. First, they show that in at least some species the cells resulting from division of the egg are not determined, or not irreversibly determined,

in early stages as particular embryonic regions or parts. Second, the fact that embryos may develop not only from the egg but from other cells of the embryo sac shows that the distinctive characteristics of the egg are not essential to embryonic development and also suggests more or less dedifferentiation in embryo formation from these other cells. Third, it is evident from embryo formation by diploid cells of the nucellus, cells of the gametophyte being haploid and the fertilized egg diploid, that embryo formation is independent of these chromosomal differences. Fourth, the data of polyembryony suggest that conditions in, or associated with, the embryo sac are factors in determining the embryonic type of development, whether from the egg or other cells of the gametophyte or sporophyte, and that there is usually a differential in these conditions such that the egg forms at the micropylar end of the sac; the more frequent development of embryos from nucellar tissue about the micropylar region is also suggestive in this connection.⁷

There are also indications that the micropylar region of the gametophyte is or becomes physiologically dominant, the degree or effectiveness of this dominance differing in different species and probably under different conditions. In the light of the data on polyembryony, development of a single embryo from the egg alone suggests a dominance effective in inhibiting embryonic development of other cells. The fact that in many cases of polyembryony the egg is not fertilized or, if fertilized, fails to develop or ceases development at an early stage suggests that in these cases dominance is largely lacking and that physiological isolation may be a factor in embryonic development of other gametophyte cells than the egg. If dominance of the gametophyte is slight or lacking, cells of the nucellus, usually from the micropylar region, may attain dominance and give rise to embryos. It is perhaps of some interest to note that the spermatophyte embryo resembles a bud in certain respects. It develops not from the whole egg but from one polar region of it. Is there any essential difference between bud formation and development of the embryo from the small active cells about one pole of the *Ginkgo* proembryo (Fig. 208, *B*)? According to this suggestion, the proembryo appears more nearly comparable to the animal embryo, and the plant embryo represents the first bud of the sporophyte, originating from what was primarily the high end of the gradient system of the proembryo.

And finally, in development of the gametophyte, position and polarity

⁷ For more complete data on spermatophytes see Coulter and Chamberlain, 1903, 1910; Coulter, Barnes, and Cowles, 1910; Goebel, 1922.

of the egg, and polarity of the embryo there is definite and characteristic relation of each step in the development of pattern to the pre-existing pattern. The data suggest that polembryony is associated with alteration of the physiological relations that determine the usual sequence of events.

POLARITY OF ANIMAL EGGS IN RELATION TO THEIR ENVIRONMENT

The chief axiate pattern of animal eggs, so-called "polarity," may be morphologically evident in a graded distribution, or a more or less sharply defined separation of cytoplasm and yolk or other substance, in position of nucleus or region of polar body formation, or it may be independent of all these. In some eggs it is apparently present or, under experimental conditions, persists only in the superficial cytoplasm or cortex; in others it may appear as a graded distribution of materials throughout the egg; in some eggs it is perhaps not established until the polar bodies form.

Ventrodorsality or dorsiventrality may also be visible in some eggs, at least after fertilization—for example, in the mesoplasm of the *Styela* egg (p. 577) and the gray crescent of the frog egg—and often, when it is not visible, experiment indicates its presence even before fertilization. In some forms, however, ventrodorsality becomes evident only in the course of development; and experiment does not give conclusive evidence of its presence in the unfertilized egg, though some physiological basis for it, presumably a slight regional difference of some sort, is probably present. This seems to be the condition in the sea-urchin egg. In ascidian eggs without visible dorsiventrality development of isolated egg pieces gives evidence of a dorsiventral pattern. The specific asymmetries of later stages of various animals are not usually evident in undivided eggs with methods at present available, though difference in developmental behavior may indicate that difference of some sort is present.

All lines of evidence indicate that patterns of animal eggs are relatively simple as compared with later stages, that is, they represent the most general features of developmental pattern. This general pattern apparently constitutes a sort of physiological co-ordinate system in relation to which further development of pattern takes place.

Relations of the developing oöcyte to its intraorganismic environment differ widely in different animals, and the question of conditions in the oöcyte environment as possible factors in determination of its pattern is best considered according to the character of this relation.⁸

⁸ In an extended description of oöcyte development in invertebrates Korschelt and Heider (1902) distinguish *diffuse* and *localized* egg formation and in both of these types, *solitary*

ISOLATED DEVELOPMENT

Oöcytes of certain annelids separate from the ovary at an early stage and pass their whole growth period as free cells in the coelomic fluid. The oöcyte of *Arenicola cristata*, an example of this type, is, when full grown, flattened to a shape approaching a biconvex lens, the axis of flattening apparently becoming the polar axis. The egg also differs somewhat in diameter in two directions at right angles to each other and in a plane vertical to the short axis, and these two diameters are probably parallel to the first two cleavage planes. There is no visible regional differentiation, not even localization of yolk.⁹ After separation from the ovaries the environment of these oöcytes is presumably not differential in any definite or persistent way, since the waves of peristaltic contraction of the body wall keep them almost continuously in motion. Nothing is known of origin or nature of the primary developmental pattern of this egg. For those who believe that spatial organismic pattern is an inherent property of a species-protoplasm or that it can originate in a single isolated cell without any relation to external factors, the *Arenicola* egg provides an apparent example of such origin. However, the possibility remains that the basis of pattern may be established before isolation from the ovary and that it persists because the uniform environment of the free cell provides no differential to alter it.

IMBEDDED DEVELOPMENT

Coelenterates and sponges present examples of this sort of relation between oöcyte and body, the oöcyte developing as a naked cell in the tissues of the parent body and evidently obtaining nutrition from surrounding cells or from intercellular fluids. These oöcytes are often amoeboid in earlier stages and may migrate. In the hydrozoa, oöcyte development takes place in the ectoderm; in scyphozoa and anthozoa, in the entoderm; and in sponges, in the mesogloea. Usually it is more or less localized in certain regions of the body—for example, on the radial canals or the manubrium in hydromedusae, on the mesenterial borders in anthozoa—but an ovary, as a special, definitely localized and bounded or-

development, that is, without special nutritive or other accessory cells, and *alimentary* development, with relation to nutritive cells; when nutritive cells surround the oöcyte development is *follicular*, when they are single or several in a group it is *nutrimentary*. The present consideration, being primarily concerned with the question of the possible role of conditions of oöcyte development in determining pattern, employs a somewhat different classification of developmental types.

⁹ Child, 1900; *A. marina*, Child, 1898.

gan, is not present. In many, if not in all, of these forms the oöcyte is subjected sooner or later in its development to an environmental differential. One pole of the ectodermal hydrozoan oöcyte comes to be separated from the water only by a very thin membrane and often protrudes from the body surface as growth progresses (e.g., *Hydra*), while the other pole is deeply imbedded and close to the entoderm, presumably the chief source of nutrition. Usually the nucleus comes to lie near the outer pole (Fig. 212, A, B). In the medusa *Phialidium*, in which the growing oöcytes form a columnar epithelium along the radial canals (Fig. 212, B) and are

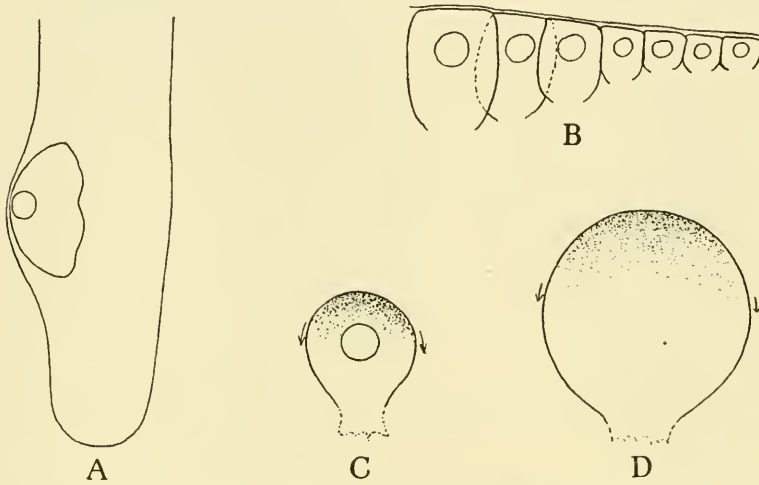


FIG. 212, A-D.—Hydrozoan oöcytes. A, oöcyte of *Corymorpha* in manubrium of medusa bud with nucleus close to outer pole; B, *Phialidium* oöcytes, forming columnar epithelium; C, D, gradient of *Phialidium* oöcyte (from Child, 1925a).

attached by their inner poles, it has been shown that a gradient, indicated by susceptibility and by reduction, is present in the advanced oöcyte (Fig. 212, C, D) and that the outer pole becomes the apical pole of the embryo (Child, 1925a). The data suggest a gradient determined by greater respiratory exchange at the outer, and uptake of nutrition at the inner, pole. There may be two opposed overlapping gradients in concentration of different substances, but the data available indicate one activity gradient decreasing basipetally. Entodermal oöcytes of some scyphozoa and anthozoa attain somewhat similar relations to the coelenteric cavity. In certain scyphozoa the entodermal epithelium in contact with a region of the growing oöcyte becomes thickened, forming a “cell crown” regarded as nutritive in function, and the oöcyte nucleus is at this pole (O. und R.

Hertwig, 1880). The *Phialidium* oöcytes are almost pedunculate (Fig. 212, C, D), and the oöcytes of some anthozoa do become pedunculate at relatively early stages.

PEDUNCULATE DEVELOPMENT

Oöcytes of many invertebrates become pedunculate sooner or later in the growth period. The peduncle may develop from a regional attachment of the oöcyte itself or from follicular or other cells. The oöcytes

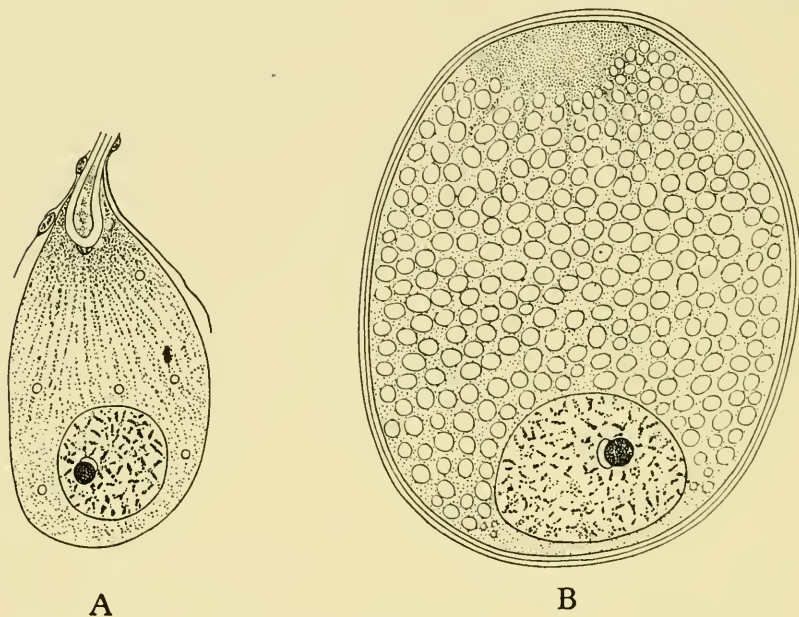


FIG. 213, A, B.—Early and full-grown oöcyte of *Sternaspis scutata*, the early stage showing the blood vessel at the attached pole (from Child, 1915b).

are primarily cells of an epithelium; development of a peduncle is associated with their growth and protrusion into the ovarian cavity. An extreme case of pedunculate development appears in the geophyrean *Sternaspis*. The peduncle attains considerable length, and a blood vessel extends throughout its length, forming a loop in the cytoplasm of the attached pole of the oöcyte (Fig. 213, A). From an early stage on, the nucleus lies at the free pole (Fig. 213, B), the polar bodies form there, and it becomes the apical pole of an embryo with spiral cleavage.

Echinoderm oöcytes show various degrees of peduncle development, ranging from regional attachment with little elongation to peduncles of considerable length. In the case of the sea urchin the attached pole be-

comes the apical pole of the embryo, according to Boveri (1901*a*, *b*); the free pole is apical, according to Jenkinson (1911*b*). Recently the finding of maturation spindles at the free poles in two species seems to have settled this question in favor of the free pole as apical (Tennent, 1931; Lindahl, 1932*b*). There was a similar difference of opinion concerning holothurians, but here also it has been found that the free pole becomes apical.¹⁰

The nucleus of the asteroid oöcyte usually comes to lie close to the surface of the cell at some region nearer the free than the attached pole, but without other definite relation to the free pole.¹¹ Polar bodies form in *Asterias* in the region where the nucleus is nearest the cell surface

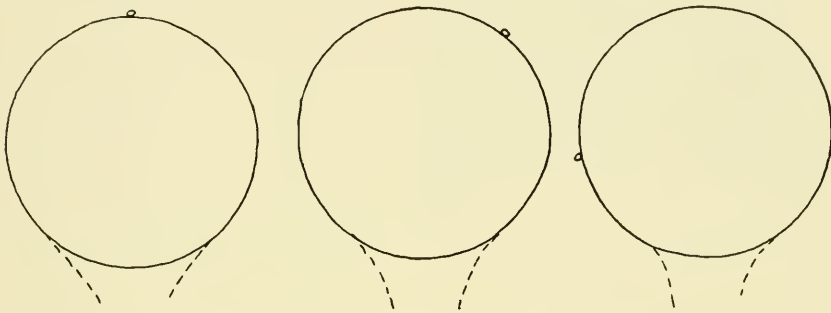


FIG. 214.—Positions of polar body in attached eggs of the starfish *Patiria miniata* (from Child, 1936*a*).

(Wilson and Mathews, 1895). According to Yatsu (1910*a*), eggs in which free and attached poles can be distinguished by shape of the egg and presence of follicular membrane invariably form polar bodies halfway between the equator and the rounded free pole. Polar-body formation in *Patiria* eggs still attached shows no constant relation to attached or free pole but depends entirely on position of the nucleus, and this appears to be determined by chance conditions, except that it is commonly nearer the free than the attached pole and may sometimes be at the free pole (Fig. 214). The differential between attached and free pole may be less in the starfish than in the sea urchin, or the nucleus may be less sensitive to it, or it may be that the nucleus comes to lie near the surface where the oöcytes are less closely packed together and there is more circulation of fluid. On the other hand, it is possible that the nucleus lies where it

¹⁰ Gerould, 1896; Théel, 1901; Oshima, 1921; Inaba, 1930.

¹¹ *Asterias forbesii*, *Patiria miniata*, author's observations.

happens to be pushed by the accumulation of substance during growth and that, if a polarity is determined by the differential between free and attached pole, it is more or less completely obliterated by the activation associated with polar-body formation. At the time of maturation a distinct dye-reduction gradient, decreasing from the region of polar-body formation, appears, primarily in the egg cortex (Child, 1936a).

Regional ovarian attachment of the oöcyte, often with development of a peduncle, is characteristic of many other animals, perhaps most commonly among mollusks and annelids; and, as far as the relation has been determined, the free pole becomes apical in most of these.¹²

Oöcytes of *Ascaris megalocephala* are regionally attached to a rhachis extending through the tubular ovary, the attached pole being more or less pointed, the free pole rounded; but they usually become spherical before maturation, and attached and free poles are not distinguishable. Moreover, position of the second polar body, the only one adhering to the egg surface, varies in relation to plane of cleavage, whether because position of formation is variable or because its position changes after formation. Also, giant eggs formed by fusion of two may develop normally. Zur Strassen (1906) and Boveri (1910a, b) believed that polarity in these eggs is finally established late, after maturation or shortly before first cleavage. Recently, however, it has been found that in certain eggs with pear-shaped shells, presumably retaining the form of the ovarian oöcyte, polar-body formation usually occurs at the blunt pole, the free pole of the oöcyte, but may occur elsewhere. Moreover, the region of polar-body formation, even when not at the free pole of the oöcyte, may apparently sometimes become the animal pole. These observations suggest that the free pole normally becomes the animal pole and determines polar-body formation there but that, when presumably abnormal conditions determine polar-body formation elsewhere, the ovarian polarity may be obliterated and a new polarity determined in relation to maturation.¹³

A multicellular peduncle develops from epithelial cells of the ovary in *Limulus* (Munson, 1898) and in arachnids (Balbiani, 1873). According to Conklin (1932), the oöcyte of *Amphioxus* and probably the ascidian oöcyte are attached by the pole which becomes apical or animal.

¹² E.g., the free pole is apical in the nemertean *Cerebratulus* (C. B. Wilson, 1899; E. B. Wilson, 1903); in the mollusks *Cyclas* (Stauffer, 1893), *Unio* (F. R. Lillie, 1895), *Musculium* (Okada, 1935), and *Dentalium* (E. B. Wilson, 1904); in the annelid *Chaetopterus* (F. R. Lillie, 1906), and in *Sagitta* (Stevens, 1904).

¹³ Schliep, 1924; 1929, pp. 230-33. See also Boveri, 1910b.

In general, the peduncle probably plays a part in the transport of nutritive material to the cell. The oöcyte often shows a special structure radiating from it (Fig. 213, *A*), but among invertebrates the nucleus often lies nearer the free pole. Respiratory exchange and elimination of products may be more rapid there. In forms with a well-developed circulatory system and with oxygen supplied chiefly by the blood respiratory exchange may perhaps be greater at the attached pole. At any rate, conditions are doubtless more or less different at free and attached poles; that these differences may determine egg polarity in many cases is indicated by the evidence.

The oöcyte of the frog is suspended from the ovarian wall in an epithelial sac, the theca; beneath that is a thin follicular membrane. The theca forms a short peduncle, an artery and vein develop in it, and a much branched capillary circulation surrounds completely the developing egg cell. In 75–80 per cent of full-grown oöcytes taken at random from different ovarian regions the boundary between pigmented and unpigmented zones is within 20° of the peduncle. Injection of the vessels with colored masses and direct observation of movement of corpuscles shows that usually the greater part of the arterial circulation is over the pigmented hemisphere (Fig. 215). There is, however, considerable variation, and cases have been found in which the arterial circulation is largely over the unpigmented hemisphere (Bellamy, 1919, 1921). Nevertheless, the high frequency of close correspondence between arterial circulation and the region of the oöcyte in which pigment develops, the apical or animal hemisphere, is far above probability. Moreover, it is probable that full-grown oöcytes may change position in the theca in consequence of mechanical conditions resulting from the ovarian contractions or other movements of the animal, for they are not in any way attached to it. Bellamy has also suggested the possibility that the circulation may change

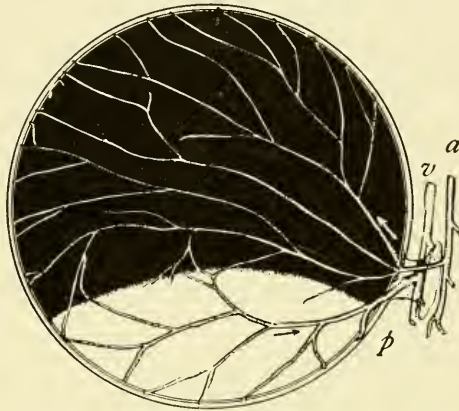


FIG. 215.—Full-grown ovarian oöcyte of frog (*Rana pipiens*), showing the usual relation between arterial and venous circulation in the theca and pigmented and unpigmented regions (from Bellamy, 1919).

during the development.¹⁴ The possibility that the "most arterial" region where the artery enters the follicle and begins to branch becomes dorsal may be noted. A study of the development of circulation in relation to pigment development in earlier stages is needed to clear up the question. Meanwhile it appears probable that polarity of the frog egg is determined by the circulation differential.

FOLLICULAR DEVELOPMENT

Both imbedded and pedunculate types of oöcytes in many animals are surrounded by a follicle, at least in early stages of growth. Cells apparently nutritive in function aggregate about the oöcytes of certain sponges; epithelial cells may be stretched into a thin follicular membrane which ruptures and disappears sooner or later as the oöcyte grows (Fig. 213, *A*; also various echinoderms), or may persist until it is full grown (holothurians). The temporary follicular membranes probably have little or no special function in some forms but are merely cells so situated that they are stretched into a membrane by growth of the oöcyte. In some groups, however—for example, cephalopods, some insects, and chordates—the follicle becomes an epithelium of considerable thickness, consisting of a single cell layer or, as in most chordates, several cells thick. Since it surrounds the oöcyte, this must obtain nutritive material from or through it. The follicular epithelium of cephalopods develops numerous folds which grow into the cytoplasm of the oöcyte. From the ascidian follicle the "test cells" pass into the oöcyte. The follicle may also secrete a chorion. Since the more highly developed follicles usually surround the oöcyte uniformly, they apparently do not provide an environmental differential. The growing oöcytes of chordates within their follicles usually approach and protrude from the surface of the ovary, and in many forms the follicle becomes more or less pedunculate. This course of development may subject the oöcyte to a differential between ovary and body cavity. The possibility that the follicular circulation may provide a differential determining polarity in the case of the frog egg was noted above. The

¹⁴ Bellamy has sometimes been cited as having abandoned, in the second paper (1921), the view advanced earlier that polarity might be determined in relation to the circulation. He did retract his earlier statement that "in every case observed the greater part of the arterial blood supply was restricted to the pigmented hemisphere," because he found some cases in which this was not true; but he emphasized the point that these concern only full-grown or nearly full-grown oöcytes and that they do not affect the earlier statement that the peduncle is within 20° of the equator in 75–80 per cent of full-grown eggs with arterial circulation largely over the pigmented hemisphere.

follicular epithelium surrounding each oöcyte in insect ovarian tubules is probably a part of a longitudinal gradient in the whole tubule.

DEVELOPMENT WITH ACCESSORY OR NUTRITIVE CELLS

Oöcytes of many animals grow at the expense of other cells, the so-called "accessory" or "nutritive" cells. These may be more or less completely resorbed during growth of the oöcyte. Very commonly the accessory cells are from the same region of the ovary as the oöcyte and are usually believed to possess the same potentialities as the cell that becomes an egg. If this is the case, some factor in the environment or relations to each other of the cells must determine their respective fates as oöcytes or accessory cells. Nutritive cells vary from one to a considerable number for each oöcyte, but number and position in relation to the oöcyte are usually more or less definite for the species.

Association of oöcytes and accessory cells is frequent among polychete annelids. The oöcyte of *Ophryotrocha* is accompanied by a single cell, larger than the oöcyte in early stages (Fig. 216, *A, B*).¹⁵ The paired cells separate from the ovary and become free in the body cavity at an early stage. Braem concludes that cells exposed to body fluid at the surface of the ovary become accessory cells, each serving as a source of nutrition to the cell immediately beneath it; the two cells become attached to each other, the one originally below the ovarian surface becoming oöcyte. In the single observed case of polar-body formation with attached nutritive cell, this cell was at the basal pole (Braem, 1894).

The primitive germ cells of the polychete *Diopatra* migrate from the primary into a secondary ovary; there they divide to form chains, consisting of a variable number of cells. In general, cell size decreases from the middle toward both ends of the chain; one cell of the middle region becomes larger than the others, protrudes from one side of the chain, and develops as oöcyte, all other cells of the chain being nutritive (Fig. 216, *C, D*). As the oöcyte grows with progressive ingestion of nutritive cells, it protrudes increasingly from one side of the chain, so that finally remaining nutritive cells of both terminal portions of the chain come to lie together at one pole (Fig. 216, *E*); this becomes the basal pole (Lieber, 1931). Apparently there is some degree of physiological integration in the chain; the gradation in cell size from the middle suggests a differential of some sort in both directions as a possible factor determining which cell

¹⁵ Braem, 1894; Korschelt, 1894.

becomes oöcyte. The data give no evidence for or against predetermination of the region of the oöcyte which protrudes from the chain. Appar-

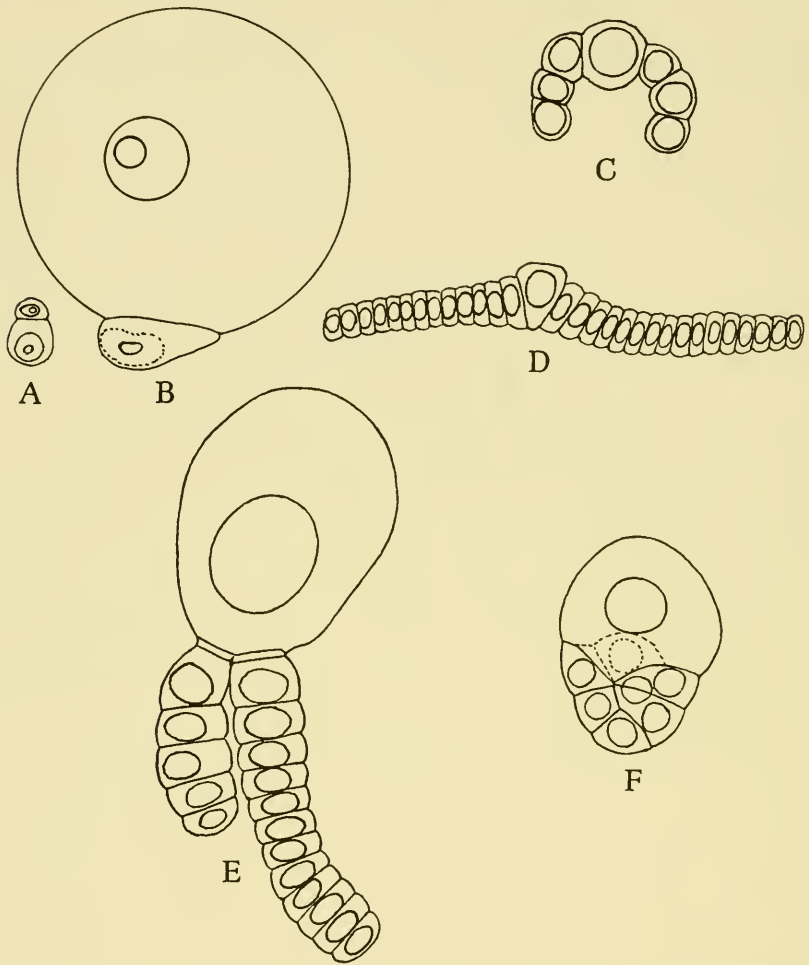


FIG. 216, A-F.—Association of oöcytes and nurse cells in certain annelids. A, B, early and late stage of oöcyte development in *Ophryotrocha puerilis* (after Braem, 1894); C, D, E, early, more advanced, and late stages of oöcyte and accessory cells of *Diopatra* (after Liever, 1931); F, eight-cell group (one oöcyte, seven accessory cells) of *Tomopteris* (after Chun, 1888).

ently it may be on the convex side of a slight curvature or be determined by mechanical relations between the cells. If this is the case, direction of the polar axis in the oöcyte is a matter of chance. Two oöcytes occasionally develop in a chain but are separated by a number of nutritive

cells, as if each, or the region of the chain in which it formed, were to some degree dominant over a certain length of chain. Somewhat similar cell chains develop in ovaries of certain parasitic copepods.¹⁶ In some of these the oöcyte develops at one end of the chain; and when it separates, the next cell becomes an oöcyte, etc. In others the oöcyte develops elsewhere in the chain, but whether polarity is determined in relation to the accessory cells seems not to be known.

Two opposite regions of the *Myzostoma* oöcyte, each associated with a single nurse cell which is completely incorporated at an early stage, are believed to become respectively apical and basal poles (Wheeler, 1896, 1897). Whether position of the accessory cells results from a pre-existing polarity of the oöcyte or oögonium or determines polarity is not clear. If the nurse cells are alike, as they appear to be, their association with the two poles of a predetermined heteropolar pattern is puzzling; on the other hand, how they can determine a heteropolar axis if they are alike is not evident. If they differ from each other, the question of the origin of these differences arises. Moreover, they are sometimes together, and occasionally there is only one.

In another annelid, *Tomopteris elegans*, groups of eight cells each, at first all of the same size and similar in appearance, occur in the ovary and later become free in the body cavity. One cell increases in size and becomes the oöcyte; seven remain accessory cells (Fig. 216, *F*) but are apparently not incorporated into the oöcyte. Presumably each group develops from a single ovarian cell; but, in any case, development of one cell as the oöcyte must be determined by a pattern of some sort in the group with dominance of this cell. The origin and nature of this pattern and the relation of egg polarity to it remain to be determined.

In the oögenesis of the geophyean *Bonellia* thin follicles are formed about groups of cells at the surface of the ovary, become pedunculate, the inclosed cells increase to a considerable number, a single cell at the proximal end of the follicle nearest the peduncle becomes oöcyte, and all others form a group at its distal pole. Since all cells of a follicle are apparently alike in early stages, a differential must be present in the follicle to determine the proximal cell as oöcyte. With the follicular peduncle at one pole and the group of accessory cells at the other the oöcyte is probably exposed to a differential adequate to determine its polarity.

Association of oöcytes with nutritive cells is very common among arthropods. In the genesis of the parthenogenetic egg of the daphnid *Sida*

¹⁶ See, e.g., van Beneden, 1870; Kerschner, 1879; Giesbrecht, 1882.

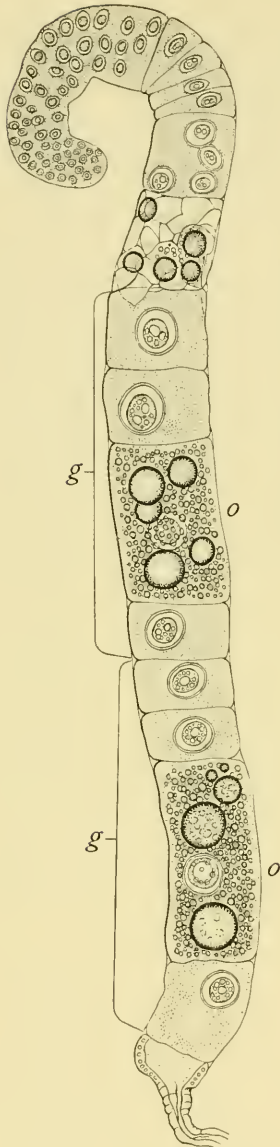


FIG. 217.—Ovarian tubule of daphnid, *Sida crystallina*, showing stage in genesis of parthenogenetic egg. In each group of four cells, *g*, the third from the apical end of the ovary becomes an oöcyte, *o*; the others are nutritive cells (from Weismann, 1877, reproduced in Child, 1915b)

crystallina, as described by Weismann (1877), the primitive germ cells of the apical ovarian region become successive series of four cells each, the third cell from the apical end in each becoming oöcyte, the others nutritive cells (Fig. 217). How differentiation of oöcyte and nutritive cells is determined does not appear. The larger winter egg, containing more yolk when full grown, uses several series of cells in its growth, sometimes as many as twelve. In *Polyphemus* the relation is different. All nutritive cells associated with a single oöcyte are at one pole of the latter, but this may be either pole as regards relation to the ovarian tubule. Polar-body formation at the pole without nutritive cells indicates that the other pole becomes basal whatever its position with respect to the ovary (Kühn, 1912).

In many insects oöcytes and nutritive cells develop from the primitive ovarian cells, all apparently alike in early stages, with great variation in number of nutritive cells associated with each oöcyte. In other species an epithelial follicle is in direct contact with the entire surface of the oöcyte, other nutritive cells being absent. For convenience in description the end of the insect ovarian tubule containing the primitive germ cells is regarded as apical, the other end as basal. From early stages cells in the ovarian tubule of the earwig *Forficula* are associated in pairs (Korschelt, 1891). The basal cell of each pair becomes oöcyte; the other, nutritive cell, develops a large branched nucleus (Fig. 218, *A*). The relation between oöcyte and nutritive cells in an aphid, *Melanoxanthum*, is shown for the parthenogenetic egg in Figure 218, *B*, and for the winter egg, requiring fertilization, in Figure 218, *C*. A protoplasmic strand connects oöcyte

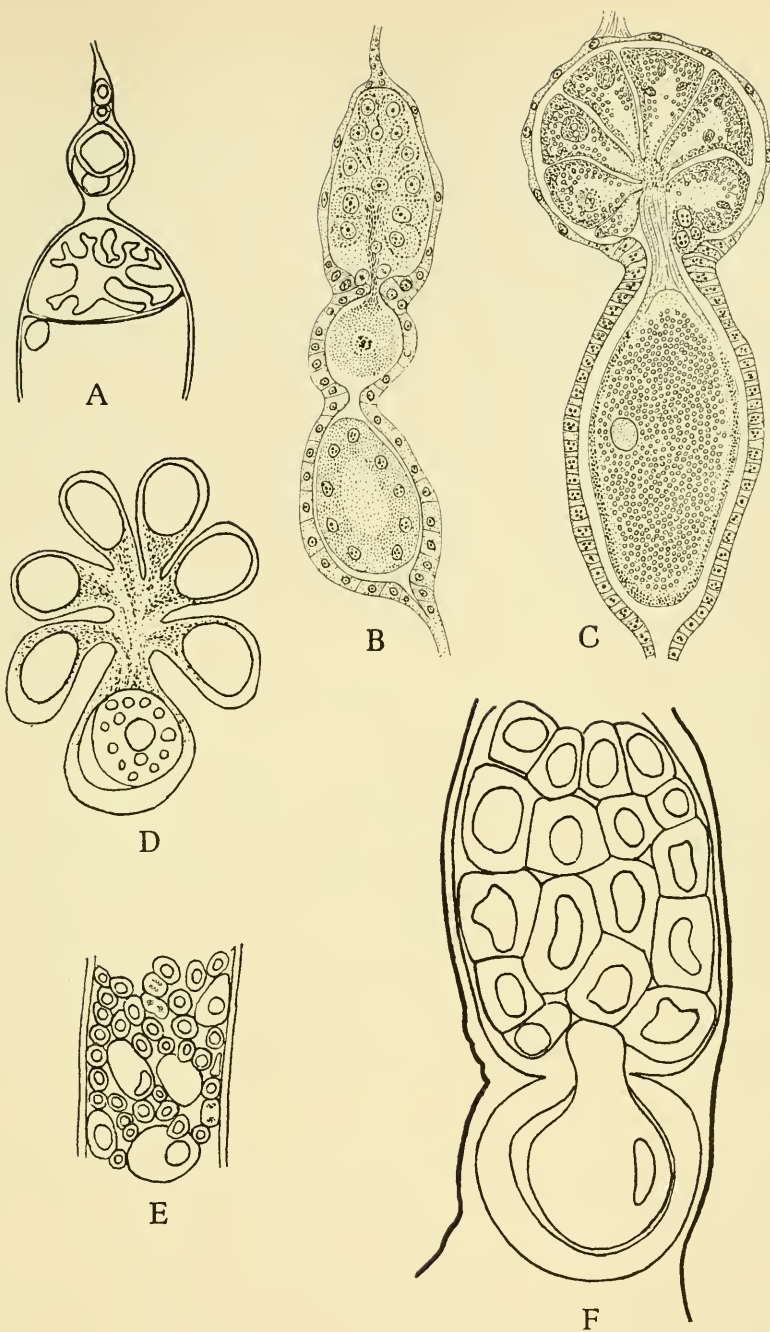


FIG. 218, A-F.—Oöcytes and nutritive cells in insects. A, the earwig *Forficula*; one oöcyte and one nutritive cell, the latter apical, in each group; nucleus of nutritive cell becomes highly branched (after Korschelt, 1891). B, parthenogenetic, and C, winter, oöcyte of plant louse, *Melanoxanthum*, connected with nutritive cells by protoplasmic strand (from Tannreuther, 1907, reproduced in Child, 1915b). D, *Dytiscus*; oöcyte with intranuclear ring and seven of the fifteen nutritive cells all cytoplasmically continuous (after Nusbaum-Hilarowicz, 1918). E, early, and F, later, stage of oöcyte development in queen bee (schematized after Paulcke, 1901).

and nutritive cells. Other Hemiptera show somewhat similar relations: in some, each of several oöcytes is connected with a different nutritive chamber apical to it, the strands from the more distant, older oöcytes passing to one side of those farther apical and younger.

In the beetle *Dytiscus* four successive divisions of an ovarian cell produce sixteen cells, the basal, larger cell becoming the oöcyte, the others the nutritive cells. The nuclear content of the primary oögonium separates into two visibly different portions preceding the first of the four divisions. From one of these chromosomes form; the other passes entire into the larger cell of the first division, and in each successive division it passes into one cell, finally forming a large ring in the nucleus of the oöcyte. This has been regarded as a kind of diminution of chromatin in the nutritive cells, analogous to that in *Ascaris*. A similar "diminution" has been described for certain other insects.¹⁷

In Figure 218, *D*, an oöcyte of *Dytiscus* is shown with the intranuclear ring and seven of the fifteen nutritive cells, all cytoplasmically continuous. What Nusbaum-Hilarowicz (1918) regards as streams of mitochondria passing from the nutritive cells to the oöcyte are indicated in the figure.

The apical region of the ovarian tubule of the queen bee contains small cells, all apparently alike; lower in the tubule the oöcytes, apparently irregularly distributed, become distinguishable by their larger size (Fig. 218, *E*). With progress down the tubule they gradually become more regularly arranged along the tubule axis and spaced at more regular intervals with the small cells between them. At a still later stage each oöcyte is associated with, and extends into, a group of nutritive cells apical to it (Fig. 218, *F*). A follicle develops about this complex, forming a thick columnar epithelium about the oöcyte, thinner about the nutritive cells (Paulcke, 1901).

The polarity of the insect egg coincides in direction with the axis of the ovarian tubule, the apical pole being directed toward the apical end of the tubule. When nutritive cells are present, the oöcyte is usually associated with a cell or group apical to it, that is, nutritive material from these cells enters through that part which becomes the apical pole. In the Crustacea, however, cells basal, as well as apical, to an oöcyte function as its nutritive cells. If the cells are all alike primarily—all potentially

¹⁷ *Dytiscus*: Giardina, 1901; Debaisieux, 1909; Nusbaum-Hilarowicz, 1918. *Gryllus*: Buchner, 1909. *Colymbetes* (Coleoptera): Günthert, 1910. See also E. B. Wilson, 1925, p. 326.

oöcytes, as is generally agreed—it may be a matter of chance in some species, perhaps in the bee (Fig. 218, *E*), which become oöcytes, which nutritive cells. However, in forms such as *Forficula* (Fig. 218, *A*), in which the basal cell of a pair becomes the oöcyte, it is evidently not chance but a definite spatial relation of some sort that determines the difference. Also, the definitely directed association of oöcyte with a cell or a group of cells apical to it is not a matter of chance.

Recent observations on differential reduction in ovaries of *Drosophila hydei* show a basipetal gradient of decreasing rate of reduction in the ovaries as a whole and in each follicle string. The basal cell, that is, the cell at the low end of this gradient in each follicle, becomes egg, the others nutritive cells. The polar gradient of the egg is a part of the ovarian and follicular gradient. A gradient from the ovarian surface inward corresponds to the ventrodorsality of the egg, the ventral side being at or toward the outer ovarian surface (see also p. 144; Child, unpublished).

EGG POLARITY: GENERAL

Eggs of different species undergo their pre-embryonic development under widely different conditions. The polar patterns of many are obviously related to differential conditions in their environment, but our knowledge of environmental conditions affecting the developing egg cell in the parent organism is a matter of inference from observation rather than of experimental demonstration. In some forms there is apparently a differential between free and attached pole, such that the free pole becomes apical, perhaps because respiratory exchange is more rapid there, while nutritive material enters at the attached pole. In other forms the pole through which nutritive material enters becomes apical, perhaps because of a gradient in the ovarian tubule of which the oöcyte is a part, as in insects, perhaps because a circulatory system is the chief mediator of respiratory exchange as well as source of nutritive material. In still other forms there is evidently relation to a factor external to the egg cell, archegonium, gametophyte, and embryo sac in plants, nutritive cells, follicles, etc., in animals; but we have even less basis for a guess as to the nature of the effective factor. And finally, in some forms the oöcyte is isolated in a fluid medium during its development from a primitive germ cell. Developmental stages of most egg cells are so inaccessible to present experimental methods, and interest has been so largely centered in the egg cell itself, that physiological investigation of the origins of egg polarity is almost completely lacking.

In other forms of development polarity is determined by various external differentials, and polarity determined by one differential may be obliterated and a new polarity determined by another in many animals. In some cases of unicellular development polar pattern is evidently determined by direction of the last preceding mitosis. It is possible that every mitosis may determine some degree of cell polarity, perhaps evanescent, or obliterated by the next mitosis in a different direction or by a differential of one kind or another—for example, the general physiological gradient and dominance in a multicellular organism, a differential in oxygen supply, electric potential, light, etc. In the absence of an effective differential external to the cell the polarity determined by the preceding mitosis may perhaps persist and become a definitive axiate pattern. Perhaps the polarities of some eggs are initiated at the final oögonial division. In other eggs a polarity so determined may be obliterated by factors in the intraorganismic environment. In the egg of *Fucus* the original polarity, however determined, is evidently readily obliterated by various external factors (pp. 423-25). At present there seems to be no adequate ground for concluding that polarity of the egg is different in origin and nature from other physiological polarities of cells and multicellular systems.

SYMMETRIES AND ASYMMETRIES OF EMBRYONIC DEVELOPMENT

The features of developmental pattern commonly distinguished as symmetries and asymmetries from polarity have often been regarded as polarities in other directions than the chief or primary polarity. They appear in definite relations, characteristic for the species, to the polar, major, or primary axiate pattern. Organismic pattern may be radial about the polar axis, ventrodorsal or dorsiventral with a resulting bilateral pattern at right angles to the polarity; or asymmetries—lateral, spiral, or irregular and specific—may be present. In many animals a polarity is evident at or before the beginning of embryonic development; but ventrodorsality, dorsiventrality, or asymmetry becomes evident only at some later developmental stage and perhaps gradually. The question how and when the symmetries or asymmetries originate and what their physiological basis is, has been and still is the subject of much discussion and speculation. Experimental evidence indicates that the basis of symmetries and asymmetries of the earlier developmental stages of various animals is present in the egg, apparently even before fertilization, though the patterns may be experimentally alterable. The point of entrance of the

spermatozoon or its path in the egg has been regarded by some as determining the median plane in certain forms. The first cleavage plane is definitely related to the median plane and so to the symmetry pattern in some animals. It has been shown in earlier chapters that, at least in certain organisms, symmetries and asymmetries show nonspecific differential susceptibility to external agents and can be altered, obliterated, and determined in the same ways as polarity. These experimental results suggest that polarity and symmetry patterns are not fundamentally different in nature. Moreover, the fact that obliteration or reversal of various asymmetries by differential inhibition and recovery is not a specific effect of a particular agent suggests that the differences of right and left sides in the forms concerned results from a primarily quantitative physiological difference. The physiological bases of axiate patterns appearing at different developmental stages doubtless differ chemically, but as spatial patterns they may be similar. For example, the polarity of a hydroid tentacle doubtless differs chemically from the polarity of a hydroid planula, but they are both characterized by gradients which, as gradients, are closely similar. In echinoderm development the pattern of differentiation in relation to the polar axis differs from that in relation to ventrodorsality, but at certain early developmental stages an apicoventral-basidorsal differential or gradient is actually present (p. 134).

Some lateral asymmetries appear indifferently on the right or left. We regard the laterality of these as determined by incidental or chance factors, but there is still the question as to the origin and nature of the asymmetry. The asymmetry of the opercula of serpulid annelids and of chelae of various decapods is reversible experimentally by removal of the dominant member of the pair (pp. 411-13). Some others are more or less constant in their laterality for the species but may be reversed experimentally or occasionally in nature—for example, the vertebrate visceral asymmetries and various other experimental and natural cases of situs inversus. The larval coelomic asymmetry of echinoderms may be either completely obliterated or reversed experimentally. Certain spiral asymmetries, such as the coiling of gasteropods, result from differential growth on the two sides of the body, but some species usually or always coil in one direction. The question how these asymmetries originate and how their laterality is determined is still unanswered. To say that they are inherited, that genetic factors are concerned in their laterality, though, of course, true, throws no light on the physiology of their origin in development.

The recent extended consideration of the problems of symmetry and asymmetry by W. Ludwig (1932) and the data presented by Schleip (1929) show the various types of these patterns and the manner in which they become evident in development, but the attempts of these and other authors to throw light on questions of origin and nature of these features of developmental pattern, while suggestive as far as they go, are chiefly significant in indicating our lack of definite information. If polar patterns are determined by factors in ovarian environment, the question at once arises whether there are factors in the ovarian environment of the oöcyte that might determine symmetry and asymmetry.

RADIAL SYMMETRIES

Radial symmetry may be primarily nothing more than absence of any pattern except surface-interior about the polar axis. It is apparently no more than this in many blastulae, sponge larvae, hydroid planulae, post-tentaculate regions of many hydroids, and many cylindrical organs. Often, however, differences appear in radii of the same body-level as development progresses. In coelenterates, for example, certain radii may become tentacle radii; in starfishes, arm axes. Some radial patterns develop from patterns not primarily radial, and these from still earlier patterns, apparently completely radial. The radii about the polar axis of the *Corymorpha* planula are apparently alike; but the succession of tentacles differs in different individuals, as H. B. Torrey (1907) has pointed out and the writer has also observed. Tentacle development may begin with appearance of a single tentacle at some point of the periphery of the planula, a second developing later on the opposite side, still later two others, opposite and midway between the first and second. In other individuals two opposite tentacles or three equidistant or occasionally four equidistant develop simultaneously, and in still others the first two tentacles may be not opposite but both in the same half of the periphery. Moreover, the order of appearance of distal and proximal tentacles may differ in a single individual. Evidently the final radial tentacle pattern results from development of tentacles in the spaces between other tentacles at a certain body-level. The individual differences in tentacle order perhaps result from the fact that the *Corymorpha* planula does not swim but in earlier stages lies with one side in contact with the substrate and only gradually erects its apical end about the time that tentacles develop. Tentacle development may be slightly retarded on the side on contact, as it is in reconstitution of pieces; and different orders may result according as erec-

tion of the hydranth region occurs earlier or later in relation to tentacle development. Quite apart from this possible differential, there is evidence of a spatial relation in the tentacle order. If one tentacle precedes others, the second tends to develop as far as possible from it. If two appear simultaneously, they are almost always opposite, or almost opposite; and if three or four appear simultaneously, they are approximately equidistant. The observations suggest that a developing tentacle is dominant over a certain distance and that, about the periphery of the planula, not more than three or occasionally four tentacles can develop. Only as this periphery increases in size do more tentacles appear in the spaces between those already present. Evidently in this and various other hydroids the radial symmetry of the mature hydranth is not predetermined but develops gradually, often through an asymmetrical or a "dorsiventral" stage, probably determined by external conditions, from a pattern in which all radii at a given body-level are alike. In general the number of tentacles which can develop simultaneously about the periphery of a coelenterate larva probably depends on the range of tentacle dominance in relation to size of the periphery. Two different quadriradial patterns, the perradial and adradial, appear in scyphomedusae; and a third, multiradial tentacle pattern, is present in some. In certain actinians two opposite radii become different from others in mesenterial and esophageal pattern, but the tentacle pattern remains multiradial. The apparently multiradial symmetry of tentacles and mesenteries in certain others—for example, *Cerianthus*—results from a sort of ventrodorsality; that is, tentacles and mesenteries develop successively from one region of the periphery. In short, radially symmetrical patterns may result from radially asymmetrical or may become asymmetrical; or certain regions may be radial, others asymmetrical, ventrodorsal, or bilateral. How patterns like the four radii of the scyphozoa or the biradial and ventrodorsal mesenterial and esophageal patterns of anthozoa are localized about the polar axis we do not know at present.

VENTRODORSALITY AND DORSIVENTRALITY

In many animals ventrodorsal or dorsiventral pattern appears first during the course of development as a graded differential in condition vertical to the polar gradient. Bilaterality is, at least in most forms, merely an expression of this apparently secondary pattern. Bilaterality, as well as polarity, is determined in the ovary, according to E. B. Wilson (1925, pp. 1021-25), but he does not suggest how it originates. If it is so deter-

mined, there must be factors in ovarian environment that can determine a second physiological axis at right angles to the polar axis. Is there evidence or probability of such factors?

Oöcytes of many animals are primarily cells of an epithelium. If this epithelium is a part of the polar body gradient, as it is in various coelenterates, or if it develops progressively, as do the lobules of a branching ovary or the ovarian rhachis and its epithelium in *Ascaris*, there is in it a graded differential, at least during its development. This is parallel to its surface, and the small fraction of this gradient in the oöcyte is vertical to a polar gradient between the free and the attached pole. Such a gradient derived from the germinal epithelium may perhaps constitute the basis of ventrodorsal pattern in some forms. Since the differential in the single oöcyte is very slight, the secondary axiate pattern may not be evident at the beginning of development and may appear gradually, as it does in many animals. Even the tridimensional pattern of the *Arenicola* egg (p. 658) may conceivably be determined in this way. The ovaries develop in lateral regions of the body, and their cells share in anteroposterior and ventrodorsal gradients of the body wall. If slight differentials in the directions of these gradients are established in the oöcyte at different stages and a third arises between free and attached poles before the young oöcyte is isolated in the body cavity, a basis for the triaxiate pattern is present.

The ovarian tubules of insects and some other arthropods presumably share, during their development, in the ventrodorsal gradient of the body. If this is the case, the ventrodorsality of the egg may perhaps represent the persistence and further development of the fraction of this gradient in the cell. Lateral asymmetries of some forms may originate in similar manner from a differential in the parent body, involving the oöcyte. Certain dorsiventralities and asymmetries of later development seem to originate in this way. The temporary dorsiventrality of the *Pelmatohydra* bud appears to be an expression of the longitudinal gradient of the parent body (p. 634). The anteroposterior and dorsiventral patterns of the amphibian limb are apparently expressions in it of anteroposterior and dorsiventral body gradients, and up to a certain stage its dorsiventrality can be altered by altering orientation of the limb bud to the body gradients. Probably patterns of various other appendages of other animals are similarly related to general body gradients. Dorsiventrality in embryos of fishes, reptiles, and birds coincides in direction with the primary polarity of the egg but may perhaps originate in the differential between surface

and interior of the blastoderm; this is perhaps also true for the mammals. Differential dye reduction in *Drosophila* ovaries suggests that in this form ventrodorsality of the egg may be determined by a differential between surface and interior of the ovary itself (p. 144).

SYMMETRIES AND ASYMMETRIES IN ECHINODERMS

Echinoderm development presents perhaps the most remarkable sequence of symmetries and asymmetries of any animal group. The early embryonic stages of most echinoderms appear to be completely radial, though ventrodorsality is indicated in some species in early cleavage or even before cleavage.¹⁸ Various lines of experiment also give evidence of a ventrodorsal gradient in early stages and of a right-left gradient in the apical archenteron.¹⁹ Development of defective skeletons in plutei from isolated 1/2 blastomeres led Plough (1927, 1929) to postulate localization at or before first cleavage, of skeleton-forming material in the basal part of the egg, excentric in the ventrodorsal axis and bilateral. However, according to W. Marx (1931), skeletal defects of larvae from early blastomeres result from ectodermal conditions. An ectodermal bilaterality, present at or before the two-cell stage, consists of two regions conceived essentially as gradient systems, decreasing from centers of highest potency. These, rather than localization of skeleton-forming material, determine bilateral aggregation of mesenchyme and so the skeletal bilaterality; in partial forms from isolated blastomeres this bilaterality is defective. This conclusion is in accord with earlier work on the relation of skeletal localization to ectoderm. Skeleton-forming material is localized in the most basal region of the unfertilized *Arbacia* egg, according to Hörstadius (1937a), not between the nucleus and center of the egg (Harnly, 1926).

The question of the relation of first and second cleavage planes to the median plane of the larva has been investigated in a number of echinoderms by intravital staining of one cell of the two-cell stage with Nile blue sulphate.²⁰ This procedure shows, in *Echinus*, *Parechinus*, and *Paracentrotus*, 50–59 per cent coincidence of first-cleavage plane and median plane; 17–42 per cent with first cleavage plane frontal, that is, at right angles to the median plane; and the remainder mostly oblique, with small percentages uncertain. In the clypeastroid *Echinocyamus* the first plane is usually frontal. In the asteroid *Astropecten* it is usually median (41.1 per

¹⁸ J. Runnström, 1920; J. und S. Runnström, 1920.

¹⁹ See pp. 134–36, 219–21.

²⁰ Von Ubisch, 1925a; Runnström, 1926b; Hörstadius, 1928b.

cent) or frontal (44.6 per cent), rarely oblique (3.6 per cent), and sometimes equatorial (10.7 per cent). In *Asterias* it is frontal. The eggs of the holothurians *Cucumaria* and *Psolus* possess a distinguishable ventrodorsal pattern and are ventrodorsally elongated; the first cleavage plane is frontal and in later cleavages a ventrodorsal difference in cell size appears (J. und S. Runnström, 1920). These data suggest a difference in degree of development of ventrodorsality at the time of early cleavage in different echinoderms and perhaps also in different individuals of the same species. In the regular sea urchins investigated ventrodorsal pattern apparently usually prevents obliquity of the first two planes, and the first plane is more often median than frontal; but in the holothurians the ventrodorsal pattern, or perhaps merely the ventrodorsal elongation of the egg, determines orientation of the first cleavage spindle in the long axis, and a frontal cleavage plane results. In general, both observation and experiment seem to indicate that ventrodorsality develops gradually, becoming an effective factor earlier in some species than in others. That ventrodorsality of the sea-urchin egg might be determined at fertilization, the meridian of sperm entrance becoming median ventral, was suggested by Jenkinson (1911b). However, if sperm enters through the micropyle at the apical pole, there is no such meridian. The physiological basis of ventrodorsal pattern is apparently present in the unfertilized eggs of some, perhaps of all, echinoderms; but conclusive evidence as to manner and time of its origin is lacking. From the blastula or early gastrula stage the larvae of sea urchins and starfishes are bilateral in appearance and show a distinct physiological ventrodorsality (pp. 134-39).

The lateral asymmetry of the hydrocoel becomes evident at different developmental stages of the coelom. In some species earlier stages of the coelom sacs appear alike on the two sides, even to formation of two hydrocoel primordia, but one degenerates later. In others asymmetry becomes evident early in coelom development. The usual position of the hydrocoel on the left side, though readily altered experimentally, indicates that genetic factors are in some way involved in the laterality; but how the asymmetry originates and how the definite spatial relation to ventrodorsality and to polarity are determined, remain to be discovered. The radial features of the adult echinoderm develop from the lateral asymmetry. The left coelom, a part of which usually becomes the hydrocoel, shows no evidence in its earlier stages of the later radial pattern. Development of the ambulacral canals from the hydrocoel probably involves spatial relations of dominance, like tentacle development; but the pat-

tern of these is primarily asymmetrical and becomes radial only secondarily.

The oral-aboral polarity of the adult echinoid, asteroid, and ophiurid is a new polarity, not coincident with that of the egg and embryo. In holothurians the original polarity persists as the longitudinal axis of the adult, and in the crinoids there is apparently a complete reversal of polarity in metamorphosis. In addition to these changes in pattern, the radial pattern of the adult becomes an anteroposterior polar pattern in clypeastroids and spatangoids; an anteroposterior motor pattern is present in different degree in some other forms; and in certain holothurians the radial pattern becomes ventrodorsal, that is, certain radii become different from others and function as ventral side. If the various echinoderm patterns originate as gradient patterns in relation either to the ovarian environment of the oöcyte or to gradient factors already present, it is evident that evolution of spatial pattern in the group has involved changes in the gradients. In this connection the approach of asteroid to echinoid larval pattern with experimental differential inhibition and of echinoid to asteroid pattern with secondary modifications of differential recovery may be recalled (pp. 208, 217).

SYMMETRIES, ASYMMETRIES, AND DETERMINATE CLEAVAGE

It was pointed out in chapter xiv that in forms with spiral cleavage the relation between the first two cleavages and the median plane is apparently constant for the species but that different authors do not agree as regards the relation in different forms. However, the species differences in size of somatoblasts, lack of coincidence of the median planes in different cells, and shifts in position of cells make it uncertain whether the apparent differences are really significant. Dorsiventrality, as indicated by difference in cell size, may be present or absent in early cleavage pattern quite independently of symmetry or asymmetry of development. It is not even certain whether a pattern of dorsiventrality or asymmetry is always present before fertilization or before cleavage. That the full-grown oöcytes of these forms possess a spatial pattern of some sort, at least a polar pattern, appears beyond question; but this pattern may be distinguishable only in somewhat eccentric position of the nucleus toward the apical pole. Beginning with the breakdown of the oöcyte nucleus, or later with polar-body formation, or still later on with fertilization, changes in visible pattern very commonly occur, consisting in localization, usually in definite relation to the polar axis, of visibly distinguishable cytoplasmic

areas, or in change in localization or distribution of areas previously visible.²¹ The form of the unfertilized egg in some species—for example, *Arenicola* (p. 658)—indicates that something more than polar pattern is present. According to Just (1912), the meridional first cleavage plane in *Nereis* usually passes through the entrance point of the sperm, and this may be any point of the egg surface. If this is true, a dorsiventral pattern determining direction of cleavage is not present in this egg before fertilization. Whether a dorsiventral pattern is present in the egg of *Chaetopterus* and some other forms before fertilization seems to be uncertain.²²

Although there is no general relation between spiral cleavage and lateral asymmetry, since most of the groups with this type of cleavage are not laterally asymmetrical, a relation apparently does exist in the laterally asymmetrical gasteropods. In most gasteropod species the coiling is dextral, with occasional sinistrally coiled individuals in some. Certain species, however, are completely sinistral with “situs inversus.” In these the cleavage is reversed, that is, cleavages dextrotropic in other forms are here leiotropic, and vice versa.²³ Observation of spiral rays in one aster of the first polar spindle in certain gasteropods led to unsuccessful attempts to correlate direction of this spiral with cleavage and direction of coiling.²⁴ Moreover, a spiral aster appears at the inner pole of the first polar spindle of the annelid *Arenicola marina* because the spindle forms equatorially and turns through an angle of approximately 90° about this pole (Child, 1898), but *Arenicola* is not laterally asymmetrical. Spiral asters are doubtless associated with protoplasmic movement, and it is, of course, possible that direction of movement is correlated with an egg pattern; but evidence of their association with gasteropod asymmetry is lacking. The suggestion by Conklin (1903*a, b*) that reversal of cleavage and of asymmetry in sinistral gasteropods results from reversal of polarity in eggs of these species after they are freed from ovarian attachment is not supported by evidence, even when position of polar-body formation is altered by centrifuging (Conklin, 1917).

The question of the manner of inheritance of sinistrality in gasteropods has received considerable attention but is not yet fully answered. The viviparous genus *Partula* produces either dextral or sinistral young ir-

²¹ See, e.g., Conklin, 1902; E. B. Wilson, 1904; F. R. Lillie, 1906; Schleip, 1914; Penners, 1922.

²² F. R. Lillie, 1906; Morgan, 1938; Morgan and Tyler, 1938.

²³ Crampton, 1894, *Physa*; Holmes, 1899, *Ancylus*, and 1900, *Planorbis*.

²⁴ Rabl, 1900; see also Schleip, 1929, pp. 111–12.

respective of its own asymmetry or may produce both dextral and sinistral individuals (Crampton, 1916, 1924). Self-fertilized individuals of *Limnaea peregra* or dextral or sinistral pairs give essentially similar results, but some difference of opinion exists as to interpretation in Mendelian terms.²⁵ Sturtevant suggested that the data indicate a simple Mendelian case with dextrality dominant but "with the nature of a given individual determined, not by its own constitution, but by that of the unreduced egg from which it arose." Later data, however, seem to require modification of this hypothesis.

The coincidence of cleavage pattern with the biradial symmetry of the ctenophore has been noted (p. 564); that a biradial pattern is present in the unfertilized egg is maintained by Fischel (1903) and some other authors, but it seems possible that the biradial pattern may develop gradually in relation to cleavage and the pre-existing polarity. According to the generally accepted view, the apicobasal axis of the *Ascaris* egg becomes the dorsiventral axis of the animal, and the anteroposterior axis becomes evident only after change in position of cells following the second cleavage; asymmetry in position of certain cells appears later (pp. 570-72). Except for the morphological polar differential usually visible in the egg and the relation of chromatin diminution to it, nothing is known concerning the pattern underlying these developmental events. The data at hand concerning entomostracan crustacea are particularly confusing. In certain of the forms studied a polar pattern is visible in the egg, but the axis of the first two cleavages is oblique to the axis of this pattern but definitely related to the median plane of the animal. In some species egg polarity and anteroposterior axis coincide; in others—for example, *Cyclops*—egg polarity is said to become dorsiventrality of later stages, with basal pole ventral.²⁶ That the basal pole should be ventral seems rather remarkable in view of the fact that in arthropods generally ventral precedes dorsal in development. Moreover, here, as in *Ascaris*, the anteroposterior polarity of the animal apparently becomes evident only secondarily, and how it is determined does not appear.

The full-grown ascidian oöcyte gives evidence of polarity in the eccentric position of the nucleus but no visible indication of cytoplasmic localization, except a surface-interior difference. As described in chapter xiv, a visible dorsiventrality results in the egg of *Styela* from cytoplasmic

²⁵ Boycott and Diver, 1923; Diver, 1925; Boycott, Diver, Hardy, and Turner, 1929; Boycott, Diver, Garstang, and Turner, 1930; Sturtevant, 1923; Crabb, 1927.

²⁶ See pp. 574-76; also Schleip, 1929, pp. 295-321 and literature cited there.

streaming, initiated by fertilization, and can be followed through the cell lineage to particular organ systems. In eggs of some other ascidians cytoplasmic localizations are not visible, but the course of development suggests that pattern is essentially similar to that of *Styela* (Conklin, 1905*a*, *b*, *c*). The cytoplasmic localizations appear to be associated with fertilization; but there is still the question whether fertilization is actually the determining, or merely an activating, factor, with localization resulting from a dorsiventral pattern already present. According to Conklin, the "mesoplasm," which gives rise to mesoderm, is a peripheral layer in the oöcyte; "ectoplasm," most of which becomes ectoderm, is in the oöcyte nucleus; and "entoplasm," later largely entodermal, is almost central. The spermatozoon enters near the basal pole, apparently not at a definite point; but its path in the egg leads Conklin to conclude that a symmetry pattern is present before fertilization.

On the basis of the character of development after fertilization of pieces of unfertilized ascidian eggs a symmetry pattern was postulated in the unfertilized egg by Dalcq (1935, chap. iii). This was regarded as consisting of two bilaterally symmetrical, crescentic areas in the peripheral cytoplasm of the equatorial region on opposite sides of the oöcyte, each extending around four-fifths or more of the circumference and overlapping each other laterally. One, the "neurochordoplasm," lies slightly deeper than the other, the "mesochymoplasm" (Fig. 219, *A*, *B*). A medio-lateral gradient pattern is also postulated in each of these crescents. Although, according to a personal communication, Professor Dalcq does not now regard these figures as representative of the true organization of the ascidian egg, they are given here with his permission because they provide, in a measure, a scheme of pattern intermediate between that postulated by Conklin and what Dalcq regards as a more adequate scheme resulting from more extended investigation (Dalcq, 1938*b*). Through the personal kindness of Professor Dalcq, which is here gratefully acknowledged, a drawing provided by him showing this later scheme is reproduced as Figure 219, *C*, and the explanation beneath the figure is given in his words. Dalcq regards the cytoplasmic movements associated with fertilization as dependent on this pre-existing pattern, but the origin of whatever pattern is present remains completely obscure. Whatever its origin and nature, the symmetry pattern seems to be a highly effective factor in development. The first cleavage plane is median, and cleavage is bilateral, as far as followed in detail, but earlier cleavage planes do not coincide with the boundaries of the cytoplasmic areas.

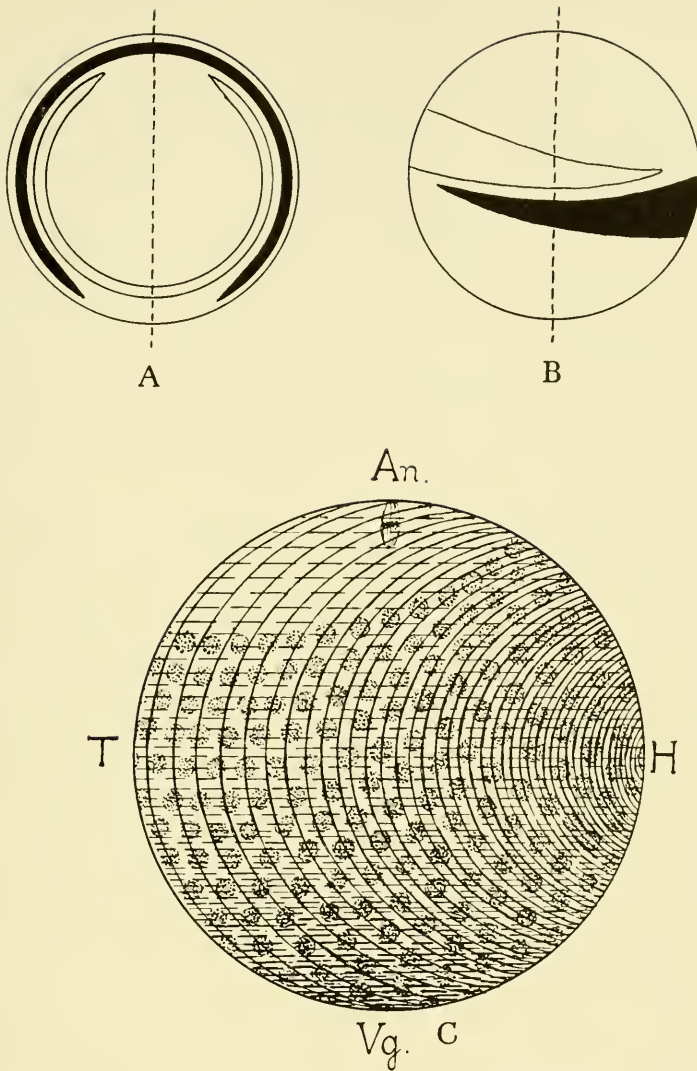


FIG. 219, A-C.—Cytoplasmic symmetry pattern of the ascidian egg, as postulated by Dalcq. *A*, *B*, scheme of pattern derived from earlier investigations: “neurochordoplasm” unshaded; “myochymoplasm” black; *A*, polar view with median plane and plane of first cleavage indicated by broken line; *B*, lateral view with polar axis and approximately the plane of second cleavage indicated by broken line (after Dalcq, *L’organisation de l’œuf chez les Chordés*, 1935). *C*, organization of the unfertilized egg, according to Dalcq’s experiments on double merogony. *An* and *Vg*, animal and vegetal poles; *H*, side of the future head; *T*, side of the future tail; the plain semicircular lines represent the postulated cortical field with its decrement from *H*; the dotted spots represent the mantle of yellow pigment lying just under the cortex; the horizontal, interrupted lines represent the internal (yolk?) gradient with its decrement from *Vg* (from a drawing provided by Professor Dalcq).

SYMMETRIES AND ASYMMETRIES IN OTHER INVERTEBRATES

Concerning origins or relations to any particular factors of developmental pattern in triclads, rhabdocoels, trematodes, cestodes, and bryozoa practically nothing is known; in the polyembryonic bryozoa the problem appears in particularly interesting form (pp. 536-37). As regards most other invertebrates, we know little more than that patterns of symmetry or asymmetry appear. In monembryonic insects polar pattern is probably derived from the ovarian tubule, but whether a ventrodorsal pattern is present in the tubule is not known. How patterns of the definitive embryos of polyembryonic insects originate is a problem for the future. Concerning possible relations of symmetry patterns of other arthropods to any particular factors, there seems to be no definite information.

AMPHIBIAN DORSIVENTRALITY

Among the eggs of vertebrates those of amphibians have been most studied with reference to the problem of origin of dorsiventrality. Eggs

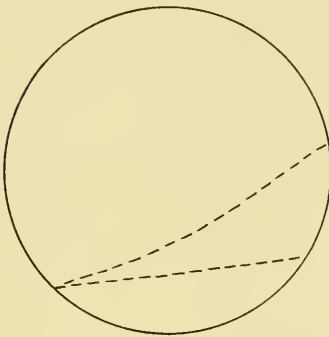


FIG. 220.—Diagrammatic outline of frog egg viewed laterally with approximate extent of one arm of gray crescent indicated by broken lines.

of most amphibians show no definite evidence of dorsiventrality preceding fertilization. In various species a part of the pigmented surface adjoining the unpigmented basal region becomes less deeply pigmented after fertilization, forming the so-called "gray crescent" (Fig. 220). The breadth of the crescent decreases bilaterally from its broadest median region; this region becomes the median dorsal region of the embryo, the median plane passing through it and the apicobasal axis. The gray crescent varies in extent and distinctness in different species, being most clearly

defined in certain anura, indistinguishable in some forms. Its median region is usually more distinct than lateral parts, and it is not sharply bounded but shades off into the more deeply pigmented and the unpigmented regions; its visible characteristics suggest a gradient system.²⁷

Before the gray crescent was recognized as a visible feature of the dorsal region, attempts had been made to determine whether a relation between point of entrance of sperm or its path in the egg and the median

²⁷ For accounts of the formation and characteristics of the crescent see Vogt, 1926*b*, 1928*b*; Bánki, 1927; Weigmann, 1927; also the general account by Schleip, 1929, pp. 558-66.

plane of the embryo existed. When the gray crescent was discovered, such a relation seemed all the more probable, since the dorsiventrality indicated by the crescent was believed to become visible only after fertilization. Experimentally localized application of sperm to the egg and histological studies of fertilization showed a high frequency of coincidence or near coincidence of median embryonic plane and plane determined by point of entrance of sperm or its path toward the female pronucleus after its entrance. Jenkinson believed, however, that other factors, gravity or light, might play a part.²⁸ The view that the spermatozoon determines or is an important factor in localizing the median plane found wide acceptance until the question was again opened by further investigation. Polyspermic eggs of *Rana fusca* were found to develop a normal gray crescent (A. Brachet, 1910a, b), and in parthenogenetic development induced by puncture a normal gray crescent and dorsiventrality develop without relation to the meridian of puncture (A. Brachet, 1911a, b; 1927). Moreover, evidence that the crescent appears in some amphibian eggs before fertilization was presented by Vogt (1926b, 1928b). Recent attempts to throw further light on the question indicate that the median plane may be determined independently of the meridian of sperm entrance.²⁹ According to Tung, entrance point of sperm is ventral, with rare exceptions, in normal development; but in dispermic eggs a definite relation between dorsiventrality and sperm entrance points does not appear. Since gray crescent and dorsiventrality appear in polyspermic and in parthenogenetic eggs, Brachet concluded that a labile dorsiventrality is present in the unfertilized egg but may be altered by the spermatozoon. Another possibility, suggested by Spemann and Falkenberg (1919) and regarded favorably by Dalcq (1935), is that the ventral region of a pre-determined dorsiventrality is a preferential region of sperm entrance. However, the earlier view that dorsiventrality is epigenetically determined by the sperm still finds support (Wintrebert, 1933a, b; 1934). At present it appears beyond question that a normal dorsiventrality and crescent may appear in parthenogenetic and polyspermic eggs of various amphibians. Moreover, the fact that, even when the fertilization meridian is in the ventral region, it is by no means always in the median plane and may be at some distance from it indicates that other factors than the sperm are concerned in determining dorsiventrality; but it does not ex-

²⁸ Newport, Ellis, and Forbes, 1854; Roux, 1887; A. Brachet, 1903, 1904; Jenkinson, 1906a, 1909.

²⁹ Bánki, 1927; Weigmann, 1927; Gilchrist, 1932; Tung, 1933; Pasteels, 1937b.

clude the possibility, suggested by Brachet, that a predetermined dorsiventrality may be altered by the sperm. It is not necessary to assume that such alteration must always result in coincidence of median plane and plane of fertilization meridian. It may only bring about more or less approximation; or, if the angle between the two is large, the sperm may have little or no effect. The possibility that a dorsiventral differential may be initiated in the oöcyte by the pattern of follicular circulation has already been noted (p. 664); this possibility is a tempting one, but it remains for the future to discover whether it is a reality.

Some recent experiments on the eggs of *R. fusca* are of particular interest here. It is well known that after removal from the uterus unfertilized amphibian eggs orient to gravity, with heavier basal pole down, as soon as the swelling of the jelly and the appearance of the perivitelline fluid permit rotation within the envelope. Some time after insemination another partial rotation begins, amounting to some 15° in certain urodeles and to 30° in *R. fusca* and some other anurans. This rotation displaces the apical pole ventrally, the basal pole dorsally, in the future median plane, and in *R. fusca* coincides in time with formation of the gray crescent.

According to studies by Ancel and Vintemberger (1933, 1935), the use of local marks shows that this "rotation of fertilization" is not rotation of the whole mass of the egg but a movement of the "pellicle," that is, of the superficial cortex over the deeper cytoplasm. On the dorsal side, movement toward the apical pole is most extensive in the future median plane and carries with it part of the underlying pigment, the region at the lower border of the pigmented hemisphere thus partially deprived of pigment becoming the gray crescent. In its median region, this is approximately 30° wide, and its width decreases laterally as the movement becomes less extensive. These authors find, however, that cortical movement is not only toward the apical pole but from lateral regions toward the median plane on the dorsal side, so that the cortex or "pellicle" becomes thicker dorsally, thinner laterally. Evidently the future dorsal region differs in some way at this stage from lateral and ventral regions, and there is a graded difference between its median and lateral regions.

In further experiment dorsiventrality and median plane have been determined experimentally in eggs of *R. fusca* by Ancel and Vintemberger (1938). Eggs removed from the uterus to a slide without water, with polar axis inclined 45° from the vertical and with basal pole upward, adhere to the slide by the jelly and are inseminated by application of sperm

to the region in contact or elsewhere as desired and then brought into water. The eggs remain in the obliquely inverted position for some 15 minutes until appearance of perivitelline fluid permits orientation to gravity by partial rotation, the basal pole rotating downward, the apical pole upward, in a vertical plane passing through the polar axis ("rotation of orientation"); that is, the heavier basal pole passes downward in the direction of its inclination from the vertical. In this orientation there is no rotation about the polar axis. The later 30° rotation of fertilization is in the opposite direction. In 87 of 100 of these eggs the median plane is within 45° of the plane of rotation of orientation, in 9 eggs it is between 45° and 90° . The gray crescent forms on the side of the egg above the basal pole as the rotation of orientation begins, and this side becomes dorsal. The same results are obtained with electrically activated, as with fertilized, eggs and with activation before or after orientation. In eggs inseminated locally at the equator with basal pole directly upward the angle between median plane and entrance point of sperm is less than 45° in 147 of 150 eggs and less than 15° in 78 of these. In these eggs the sperm is a more or less effective factor in determining dorsiventrality. When sperm and rotation of orientation act in different directions, the latter overcomes the former. The localization of dorsal regions and median planes by change in position of cytoplasm and yolk by reaction to gravity in eggs maintained in inverted and partly inverted position has already been discussed (pp. 428-30). In general, the data indicate a physiological basis for dorsiventrality in the unfertilized egg, but this is evidently alterable experimentally before the superficial movements associated with formation of the gray crescent take place.

DORSIVENTRALITY IN OTHER VERTEBRATES

In the meroblastic eggs of fishes, reptiles, and birds the longitudinal or polar axis of the embryo may probably be regarded as coinciding with an egg meridian, though it is practically at right angles to the egg axis. The dorsal side of the embryo is the side adjoining the free surface of the blastoderm; consequently, the question of symmetry becomes the question of how embryonic polarity is determined. A possible indication of axiate pattern and symmetry, consisting in a regional difference in the marginal periblast, was described by Rückert (1892). One side of the teleost blastodisc, supposedly the posterior side, is thicker than the other before cleavage.³⁰ Also, one side becomes more susceptible in early stages

³⁰ Oellacher, 1872; Agassiz and Whitman, 1885; Kowalewsky, 1886.

of some forms (p. 149), but how these differences originate is not known. If they are expressions of axiate pattern, they indicate the radius or region of initiation of axiate development, and bilaterality is apparently a secondary result of a longitudinal and dorsiventral differential in the developing region. This region is not the only one capable of forming an embryo, as experiment has shown (pp. 521-22), but is probably living a little more rapidly than other parts, so that certain events occur in it earlier than elsewhere, and its dominance determines the course of development. How it is localized is still to be discovered.

The same question, how the longitudinal embryonic axis and the median plane are determined, arises with regard to reptiles and birds. Data on reptile eggs are few: the embryonic axis of the gecko is said to be approximately at right angles to the long axis of the egg (Will, 1893). In the chick the embryonic axis is more or less nearly at right angles to the long axis of the egg shell; and, with the pointed end to the right, the anterior end of the embryo is usually directed away from the observer.³¹ This orientation, however, is by no means constant; deviation in either direction is frequent but usually not great, though inverted orientation sometimes occurs.³² The pigeon's egg shows a somewhat similar relation between long axis of the embryo and of the whole egg, but the modal angle between the two is about 70° , with variation from 8° to 135° , and inversion is rare.³³

The early oöcyte of the pigeon, according to Bartelmez, exhibits bilaterality. A polar axis is indicated by the eccentric position of the nucleus nearer one pole, the future apical pole, and by positions of cytoplasmic granules and yolk nucleus. This polar axis is "not infrequently" vertical to the surface of the ovary, with the apical pole attached. A second axis, the long axis of the oöcyte, is also indicated, and bilaterality results from position of the nucleus nearer one pole of the long axis, that is, only one plane divides the oöcyte into symmetrical halves. Whether

³¹ Von Baer's rule; von Baer, 1828.

³² Duval, 1884, found the two axes vertical to each other in 75 per cent; Kopsch, 1927, in 33 per cent; and other figures range between these—e.g., Butler, 1935, 50 per cent. According to Butler, deviation up to 45° to the right is 33.1 per cent; to the left, 10.6 per cent; more than 45° to the right and left, 2.5 per cent each. See also Féré, 1900; Rabaud, 1908. Percentages of inversions given by different authors vary widely: J. C. Dalton, 1881, 12 per cent; Duval, 1884, 0.6 per cent; Bartelmez, 1918, 33 per cent.

³³ Bartelmez, 1912, 1918. See also Blount, 1909; Patterson, 1909. Bartelmez reports 0.67 per cent of inversion in one lot of 600 eggs and 2 per cent, all in eggs of two of some 90 birds with a total of 400 eggs.

this pattern is predetermined in the oöcyte independently of ovarian environment, as Bartelmez seems to believe, or is related to some environmental factor, as seems possible, it is not evident that it has any necessary relation to bilaterality of the embryo, for the median plane of the embryo forms an angle varying from 8° to 135° from the median plane of the oöcyte, with a mode at 70° . Bartelmez also finds that in some oöcytes the shorter diameter of the nucleus is inclined to the long axis of the oöcyte by about the same angle as the embryonic axis to the long axis of the egg, and he suggests that the embryonic axis may be determined by the nucleus. He regards the polar axis as predetermined in the oöcyte, and polarity and symmetry as inherent properties of protoplasm, persisting from generation to generation. If this were the case, however, it would seem that much greater constancy of angle between embryonic axis and long axis of the egg might be expected, but the relation between the two axes gives a probability curve with less variation in eggs from one bird than in those from more than one. In short, the data concerning chick, pigeon, and other birds do not exclude the possibility that embryonic axiate pattern is not predetermined but originates in reaction to some more or less variable factor in the organismic environment of the oöcyte or egg. The possibility that its determination may be correlated in some way with position of the ovary on the left side of the body seems not to have been considered.

In mammals follicle development perhaps subjects the growing oöcyte to a definitely directed environmental differential, and a polarity may result; but that polarity is not the polarity of the embryo. Oöcyte polarity may determine polarity of the blastocyst, that is, position of the embryonic area, which represents essentially a blastoderm; but whether it plays any further role in determining developmental pattern is not certain. Since embryonic dorsiventrality develops in a definite relation to the surface-interior pattern of the embryonic area, the dorsal side being toward the outer surface, it may represent either persistence of oöcyte polarity in the embryonic area or a direct reaction to surface-interior differences or both. Since the apparently radially symmetrical blastocyst gives no evidence of factors determining direction of the longitudinal embryonic axis, the possibility suggests itself that this axis may be determined by factors in the uterine environment, or, more specifically, in the region of implantation. If a physiological differential is present in the uterine wall, subjection of the embryonic area to it on contact with, or implantation in, the uterine mucosa may perhaps determine that axis. Moreover, if

determination does occur in this way, direction of the axis in relation to the uterus as a whole may differ, more or less, according to the region with which the blastocyst becomes most closely associated. If there is a longitudinal or a dorsiventral gradient system in the uterine wall, or a gradient system in relation to each oviduct, the blastocyst adhering to, or implanted in, the mucosa is subjected to the differential in the region concerned. Even in the monotremes axial determination during the intrauterine period and in relation to intrauterine environment does not appear impossible.³⁴

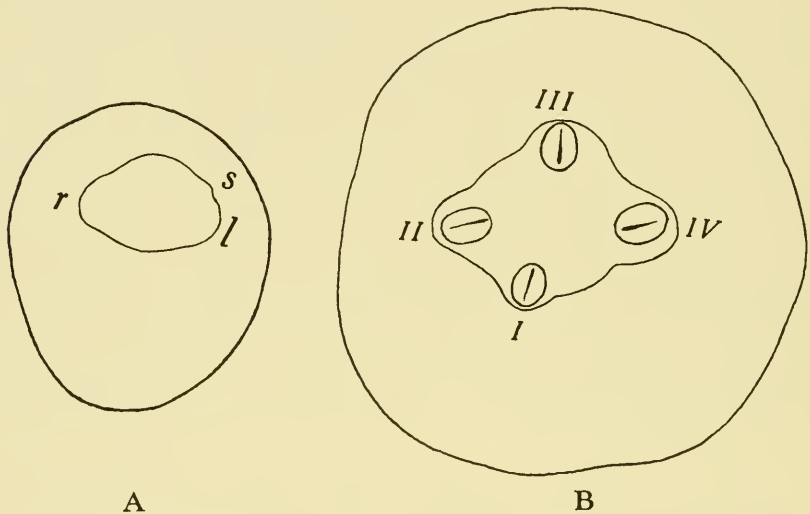


FIG. 221, A, B.—Polyembryony in the nine-banded armadillo, *Dasypus novemcinctus*. A, outline reconstruction of blastocyst and vesicle with two primary buds, *r* and *l* and probable early stage of a secondary bud at *s*. B, outline reconstruction of later blastocyst and vesicle with four embryonic primordia. *II* and *I* supposedly from one primary bud; *IV* and *III* from the other (after Patterson, 1913).

In this connection the case of the nine-banded armadillo (*Dasypus novemcinctus* = *Tatusia novemcincta*), in which four embryos develop from a single egg, requires special consideration. Here the ectodermal vesicle formed by invagination of the ectoderm of the embryonic area into the interior of the blastocyst gives rise to two "primary buds," each of which in turn gives rise to two "secondary buds"; and each of these, together with a part of the mesoderm and entoderm, becomes an embryonic pri-

³⁴ Although no complete search of the literature has been undertaken in attempting to discover evidence for or against the possibility of intrauterine determination of axiate pattern in mammals, it appears that many accounts of early mammalian development do not consider the question.

mordium. The two primary buds arise on right and left sides of the vesicle (Fig. 221, *A*), facing the openings of the Fallopian tubes (Patterson, 1912, 1913). That factors in the uterine environment are concerned in localizing the two primary buds and in orienting their opposed polarities appears more probable than that they are determined independently of environment by fissions of the blastocyst (Newman, 1917, pp. 47-49). According to Patterson, two thickenings appear on each primary bud, one at its tip, the other on its left side (s of Fig. 221, *A*), as viewed from the center of the free surface of the blastocyst. Later the four embryonic primordia appear more or less equidistant from each other (Fig. 221, *B*), two of them, *II* and *IV*, retaining the positions of the primary buds, the others, *I* and *III*, being respectively dorsal and ventral. How formation of a secondary bud on the left side of each primary bud is determined remains a problem; if it indicates an asymmetry in the primary bud, the origin of that is entirely obscure.

In another species of armadillo³⁵ tertiary buds arise in variable number, six to twelve embryos being formed (Fernandez, 1909). It has been suggested that a number of radially arranged apical points are determined in the ectodermal vesicle before visible budding and that those which happen to lie toward right and left sides of the uterus have more room for growth (Newman, 1917, pp. 50-51). This hypothesis is without support of evidence and does not seem to be required by the data of observation. Differences in rate and somewhat less uniformity in physiological dominance and isolation would permit repetition of budding more frequently in some of the primary or secondary buds than in others. Moreover, it is difficult to conceive how such a radial system of presumptive embryonic primordia could arise all at once from what Newman regards as originally a single embryo. The budding of the ectodermal vesicle results in the determination of a new longitudinal axis for each embryo and, consequently, a new bilaterality (Fig. 221, *B*); but dorsiventrality remains the same in all.

VERTEBRATE ASYMMETRIES

All vertebrates are normally asymmetrical as regards position of various organs—heart, stomach, liver, coiling of intestine, etc.—and exhibit various functional asymmetries. In general, these asymmetries are highly constant as regards laterality, but situs inversus occurs occasionally. That genetic factors are concerned in some way is evident, but how the constancy of laterality is determined is no better understood here than

³⁵ *Dasypus hybridus* = *Tatusia hybrida* = *Mulita hybrida*.

for invertebrate asymmetries. A few points that may have some bearing on the problem are briefly noted.

In both urodele and frog developmental stages with open neural folds removal and reimplantation in the same place, with anteroposterior reversal, of a dorsal rectangular piece of sufficient size from the middorsal region, including presumptive neural tissue and underlying inductor material, result in reversal of asymmetry; if the piece is merely removed, asymmetry is not reversed.³⁶ Other experiments show that altered orientation of prospective neural tissue does not influence asymmetry; consequently, it seems evident that the archenteric roof is a predominant factor at the stage concerned in determining the laterality of asymmetry, but it is not the only factor. These experiments throw no light on the origin of asymmetry.

Reversal of polarity in the undivided frog egg by reversal of position in relation to gravity does not reverse asymmetry (Hämmerling, 1927), nor does separation of right and left $1/2$ blastomeres in a urodele by gradual constriction with a hair ligature (Mangold, 1921). Gradual partial constriction by ligature of the urodele blastula in the median plane gives a somewhat different result. Monsters with more or less anterior duplication develop, the left member always with normal asymmetry, the right usually with situs inversus.³⁷ If constriction is continued to complete separation of right and left halves, the left half develops with normal asymmetry, the right about equally with normal and reversed asymmetry (Ruud und Spemann, 1922). Division of the heart primordium into right and left halves at the tail-bud stage results in development of two hearts, the left with normal, the right with reversed, asymmetry (Ekman, 1925). These data bring out two important points: first, asymmetry apparently develops gradually, for reversal of polarity in the undivided egg and separation of right and left halves at the two-cell stage do not alter asymmetry, but with separation at the blastula stage the right half is often reversed; second, the laterality of asymmetry is apparently more stable in the left than in the right half, as if there were on the left side a region dominant as regards asymmetry. A gradually developing activity gradient with high end on the left side is postulated by Huxley and De Beer (1934, pp. 73-82).

³⁶ Spemann, 1906, 1918; Pressler, 1911; R. Meyer, 1913.

³⁷ Spemann und Falkenberg, 1919. Anterior duplications in fishes, if they do not extend posteriorly beyond a certain level, often show situs inversus in the right member, as noted by Stockard, 1921, and by other authors.

In various experiments two individuals or organ systems developing side by side with cellular continuity between them very generally show opposed symmetry or asymmetry patterns. Even two hydranths of *Corymorpha* developing close together become "dorsiventral" or bilateral in relation to each other, and the mirror-imaging of amphibian appendages has long been known (pp. 390-95). Apparently there is in these and other similar cases a relation between the two members tending to make the pair a symmetrical whole. If the asymmetry of the amphibian appendage results from a physiological differential, these cases of mirror-imaging receive a simple interpretation; the high or the low side of the differential is common to both, or the differential in one may induce a differential symmetrical to it in the other.

Both morphological and physiological evidences of asymmetry appear in the chick blastoderm of the head-process stage. The anterior end of the primitive streak bends slightly to the left (Fig. 167); and in chorio-allantoic grafts from this stage pieces from the left side show greater developmental capacity than those from the right, suggesting a differential in physiological condition from left to right (Rawles, 1936). In later stages the embryo undergoes torsion progressively from the head region posteriorly, so that it finally comes to lie on the left side. This suggests a growth differential, higher on the left side during torsion, and dye reduction gives evidence of a physiological asymmetry at these stages (p. 160 and Fig. 55). Reversal of direction of this torsion (heterotaxia) occurs occasionally under natural conditions. In a brief abstract Gray, Dodds, and Worthing (1940, *Anat. Rec.*, 78, Suppl., p. 77) report 6 per cent heterotaxia in some nine hundred embryos. They also find that various optically active *d*- and *l*-substances have opposite effects on frequency of heterotaxia and suggest that the lateral reversal of differential growth is due to interference with normal metabolism or to an asymmetrical utilization of, or sensitivity to, the optically active substances.

Of the four embryos developing from a single armadillo egg, one pair is connected with the right, the other with the left, placental disk. In a study of symmetry relations, as indicated by anomalies in the integumentary bands of scutes, it was found that members of a pair are more nearly identical and exhibit interindividual mirror-imaging more often than individuals of different pairs (Newman, 1915b, 1916). Mirror-imaging in individuals of opposite pairs is interpreted by Newman as indicating the primary bilaterality of the original embryo; mirror-imaging in individuals of a pair, as indicating the secondary bilaterality of the primary bud,

which is superimposed on the primary bilaterality and more or less obliterates it; the tertiary bilaterality of each individual is superimposed on the secondary bilaterality and tends to obliterate both it and the primary. It seems possible, however, that mirror-imaging in members of a pair may be, at least in part, the expression of action on each other, like inhibition or other modification of adjoining sides in various other forms.

STRUCTURAL CONCEPTS OF DEVELOPMENTAL PATTERN

The "intimate structure" of some sort, so often postulated as the basis of organismic developmental pattern, has been regarded by some as inherent in the protoplasm concerned, by others as originating in relation to some factor external to the protoplasm. Resemblances or analogies between organismic structures, cells, and even whole organisms and crystals have been pointed out repeatedly; and hypotheses of an organismic pattern essentially crystalline, or in some way resembling crystalline structure, have appeared again and again. The discovery of fluid crystals and supposed resemblances of their behavior to that of living protoplasms seemed to give support to these views.³⁸ Observations with polarized light have given evidence of optical anisotropy in many plant and animal structures, and X-ray analysis is advancing still further our knowledge of the ultramicroscopic structure of biological materials.³⁹ However, many of the structures in which evidence of a definite molecular or micellar pattern is found are nonliving products of protoplasmic activities, such as cuticular substances, cellulose, and other nonprotoplasmic membranes—shells, skeletal structures, fibers of cellulose or protein constitution, hair, silk, etc. Evidence of ultramicroscopic orientation also appears in certain protoplasmic structures, muscle, nerve, fibrillar connective tissue, various other fibrillar structures of cells, and in the highly condensed or structurized substance of sperm heads. It is perhaps a point of considerable importance that this evidence concerns nonprotoplasmic structural products and protoplasms with a high degree of structurization. In some of these structural patterns—for example, connective tissue and bone—orientation of ultramicroscopic particles apparently represents primarily a result of mechanical environmental factors rather than a crystalline space lattice, and pressure and tension are probably concerned in many other such patterns. Evidence of change in pattern of proteins in relation

³⁸ See, e.g., O. Lehmann, 1911. Resemblances and differences between fluid crystals and organismic structures are discussed in many other papers.

³⁹ Seifriz, 1935; W. J. Schmidt, 1937; F. O. Schmitt, 1939 and citations.

to tension has been obtained by X-ray analysis.⁴⁰ There is also evidence of molecular orientation with respect to surfaces and interfaces. These mechanical and surface orientations, however, may be in all possible directions in a cell or a multicellular organism; consequently, it does not appear possible that they can constitute the basis of axiate developmental pattern. Protoplasms in general have not yet been shown to possess any such structure that might serve as a basis for developmental pattern. Evidence of the presence in eggs or other reproductive cells of a space lattice related to developmental pattern is at present lacking. The peripheral cytoplasm of the egg of a sea urchin (*Strongylocentrotus purpuratus*) and, in the centrifuged egg, the zone of clear cytoplasm become birefringent on fertilization, according to Moore and Miller (1937); but this pattern can be only secondarily associated with developmental pattern, for the egg possesses a polar, and probably a ventrodorsal, pattern before fertilization. The cytoplasm of *Amoeba* is said to be birefringent (W. J. Schmidt, 1937), but *Amoeba* has no persistent axiate pattern. Evidence of orientation of elongated molecules in relation to direction of protoplasmic flow has also been obtained in many cases.

The hypothesis that the organism is fundamentally crystalline in character has been advanced in one form or another by various authors, and similarities between organism and crystal have been pointed out repeatedly. Interpretation of the *Bruchdreifachbildungen* (see pp. 387-95) and other features of development in terms of a hypothetical space lattice has been attempted by Przibram.⁴¹ Since the space lattice is entirely hypothetical, it can, of course, be assumed to undergo the changes required to account for the experimental results, and the required assumptions are made for each case. Amphibian asymmetries and experimental reversals have suggested analogies to crystals to certain authors.⁴² The accumulating evidence for existence of greatly elongated molecules and of union of units, end to end, to form long chains—for example, in cellulose and in various proteins—has been regarded by some as affording new support for an essentially crystalline or paracrystalline basis for developmental pattern. Seifriz, for example, in recent publications, holds that there is in protoplasms a continuity of structure consisting of such elongated molecules and that polarity and symmetry of organisms result from

⁴⁰ E.g., Astbury, 1937, 1939; Astbury and Bell, 1938.

⁴¹ Przibram, 1906, 1921, and other papers.

⁴² Spemann und Falkenberg, 1919; Harrison, 1921a.

it.⁴³ However, it is a long jump from the molecular patterns of cellulose, silk, wool, etc., or from those of muscle, nerve, or connective tissue, or even those of proteins, to the polarity and symmetry of whole organisms; and the categorical statement quoted in footnote 43 certainly goes far beyond the evidence.

A fundamental asymmetry of protoplasms has been postulated by some because it has been found in many cases that only one of the two optical isomers of certain constituents of protoplasms, amino acids, various lipoids, and sugars serves for protoplasmic synthesis. If such an asymmetry is present, it evidently does not necessarily determine an asymmetric organismic pattern, for many organisms show no evidence of asymmetry. Moreover, in many of those that are asymmetric the asymmetry becomes evident only relatively late in development, only in certain organ systems or organs, and always in definite relation to more general features of pattern that are not asymmetric. If molecular factors have anything to do with organismic asymmetry, they seem to be effective only as localization of primordia and structurization progress.

If this kind of asymmetry is a general property of protoplasms and is concerned in determining organismic asymmetry, what happens when the organismic asymmetry is experimentally reversed—for example, the coelomic asymmetry of the sea-urchin larva or vertebrate asymmetry? Has the one isomere been transformed into the other? Does the individual with reversed asymmetry use the other isomere in synthesis? Does experimental obliteration of asymmetry by differential inhibition or establishment of a new asymmetry by an environmental differential result from a corresponding change in isomere pattern? These questions remain unanswered, and there is still the more general question: How can a molecular asymmetry determine differences in metabolism, in rate and amount of growth, and in differentiation on the two sides of an organism? A general asymmetry of protoplasms has not yet been optically or otherwise demonstrated; but, as pointed out in the preceding chapter, molecular factors may be concerned in many of the asymmetries of highly differentiated and structurized protoplasms.

A molecular hypothesis which attempts to account for specific regional localizations has been advanced by Harrison.⁴⁴ Calling attention to the

⁴³ Seifriz, 1935; 1936, chap. xv; 1938. In the latest of these papers he says: "Polarity of cells and symmetry of organisms exist in virtue of molecular patterns indicative of the crystalline state."

⁴⁴ 1936*b*, 1937, and suggestions concerning a molecular basis of pattern in various earlier papers.

very general belief that the specific characteristics of protoplasts result chiefly from their proteins and to the conception of protein molecules as possessing definite spatial patterns, polarity, and symmetry or asymmetry, he suggests that embryonic developmental pattern results from the configuration of protein molecules. In consequence of their bipolar character they tend to orient in the cell, "possibly with respect to the point of attachment in the ovary." Different chemical properties at the two poles bring about different reactions; and the resulting substances are carried electrophoretically toward opposite poles of the cell, forming two opposed material gradients, each decreasing in concentration from one pole to the other. Differences in concentration along these gradients, together with substances of nuclear origin, initiate new reactions locally and so begin the progressive localization and complication of development. Symmetry and asymmetry are supposedly further expressions of the molecular configuration

This hypothesis admits the possible relation of developmental pattern to environment in suggesting that the molecular orientation may occur in relation to attachment of the oöcyte, but it does not suggest how the orientation is brought about. If orientation does occur in this way, must it not be because a difference in condition between point of attachment and other regions produces a differential of some sort in the cell? If this be granted, it is probable, since the cell consists of living, metabolizing protoplasm, that this differential will involve a differential or gradient in metabolism, rate of living, or whatever we may call the continuous physicochemical change constituting life. In short, this hypothesis of Harrison's does not exclude, but seems to require, a gradient involving the essential activities of the protoplasm to bring about the molecular orientation. It is perhaps questionable whether molecular orientation ever occurs spontaneously, that is, entirely without any relation to factors outside the molecules concerned. If such a gradient in the oöcyte is a factor in molecular orientation, it, not the orientation, is the primary axiate pattern.

Origin of symmetry and asymmetry in terms of this hypothesis raises further questions. If the molecules possess a dorsiventral and bilateral or asymmetric pattern and different substances are produced on different sides, they, like the polar substances, must be transported to opposite sides of the cell, dorsally and ventrally or laterally; but how? Independent cataphoresis in two opposite directions along two or three different axes does not appear possible. All such hypotheses require postulation

of factors in addition to the molecular orientation to account for localization on the molar or regional scale characteristic of organismic pattern. In Harrison's hypothesis the essential point for developmental pattern is not the orientation of the protein molecules but the transport and localization of the substances produced toward opposite poles of an axis. An electrophoretic factor originating from a metabolic differential in relation to attachment of the oöcyte could bring about the transport whether the protein molecules were oriented or not, or might determine production of the substances *in situ*, and molecular orientation would be entirely unnecessary. In this connection it is of particular interest that an attempt to discover evidence of molecular orientation by application of X-ray analysis to living amphibian neural plate, neural tube, ear, ectoderm, myotomes, notochord, and yolk and various tissues of chick embryos is entirely without positive result (Harrison, Astbury, and Rudall, 1940, "An attempt at X-ray analysis of embryonic processes," *Jour. Exp. Zööl.*, 85). The authors conclude that the negative results do not support the theory of molecular orientation but do not disprove it.

Not only the origin of spatial organismic pattern but many other features of development are difficult to account for in terms of a primary molecular pattern. For example, how is the reconstitution of apical partial hydranths from short hydranth stem pieces under good conditions and of complete individuals under inhibiting conditions to be accounted for? How can molecular orientation determine that apical halves of early sea-urchin cleavage stages produce only ectoderm in sea water but after treatment with LiCl produce entoderm and mesenchyme? Ventrodorsality and even polarity can be obliterated in embryos or isolated pieces of various organisms by exposure to certain concentrations of inhibiting agents (chaps. v, vi). Moreover, ventrodorsality in echinoderm embryos may be obliterated without obliterating polarity. Hydroid polarity can be determined by difference in oxygen content of the water. In such determinations orientation of molecules, if it occurs, must result from the metabolic differential; also, it must be entirely independent of cell boundaries. In other words, any molecular orientation present in these and many other cases must result secondarily from the metabolic differential pattern.

The similarity between organism and crystal apparently consists chiefly in appearance of a definite spatial pattern in both; but in the crystal the pattern is geometric, while in the organism regional differences in metabolism and in concentration and kind of substances arise progressively.

To the extent that chemical reaction takes place, the crystal pattern disappears; chemical reaction is an essential feature of pattern in organisms. That organismic pattern originates in a crystalline space lattice appears on the basis of the evidence extremely improbable, but that crystalline structures and molecular orientations appear in organisms is evident, and the evidence indicates that they are secondary effects of the pattern. Some of them are evidently determined by purely local conditions and are only remotely related to the general pattern; others are more directly related to that pattern. Probably molecular or micellar orientation is very generally characteristic of fibrillar structures and of various particles, inclusions, and metabolic products. Mechanical tension and pressure may bring about orientation of molecules or particles, orientation may occur in relation to interfaces and to flow, and some protoplasts may become crystalline; but there is, at present, no evidence for, and much against, the concept of organismic pattern as primarily a pattern of molecular structure and orientation. Such concepts seem to put the cart before the horse.

Some or all of the specific asymmetries of unicellular forms, spermatozoa, etc., discussed in the preceding chapter, and perhaps many of the more minute structural features in multicellular forms, may be expressions of a molecular or crystalline structure; but it may again be emphasized that, in general, they appear to represent more nearly ends than beginnings of development and that they develop in definite relations to more general patterns of earlier stages. If a structural factor of this sort is involved in lateral asymmetries of multicellular forms, it also is apparently a derivative of developmental pattern; but the evidence available suggests that lateral asymmetry is a physiological gradient pattern.

More or less definite patterns on a molar scale appear in many inorganic systems quite independently of any molecular pattern of the whole. For example, any particular region of a flowing stream shows a definite pattern of flow of water, erosion or deposition, and morphology of banks and bed. This pattern develops from the activity of the water in relation to gravity, an environmental factor, and to the banks and bed. The molecular pattern of the water undergoes continuous change; the banks and bed may consist of various substances—sand, clay, and rock of different kinds—with many different molecular patterns, but the general morphology of the stream depends on the configuration of banks and bed and energy of flow rather than on molecular pattern of any of the stream components. In “normal” environment, that is, within certain limits of

change in conditions, the stream pattern, like organismic pattern, is highly persistent but does undergo a gradual development which is a continuous equilibration. But under "abnormal" conditions the pattern may change greatly—for example, in a flood or when dammed. That the living organism is more nearly analogous to a system of this kind than to one resulting from a particular molecular patterns seems probable.

THE VARIOUS GRADIENT CONCEPTS

The frequency with which the suggestion that physiological polarity is a gradation or gradient pattern of some sort or a stratification of substances has appeared in biological literature is both interesting and significant. A polar pattern of this sort was suggested for the sea-urchin egg and later for the egg of *Ascaris* by Boveri (1901*b*; 1910*a*, *b*). In many papers on regeneration Morgan postulated gradations of formative substances and also suggested gradation of tension. More recently Runnström and his co-workers have interpreted their experimental results on sea-urchin development in terms of two opposed substance gradients in the polar axis, another, ventrodorsal gradient, and a left-right gradient pattern (pp. 140, 241). A somewhat similar hypothesis has been advanced by von Ubisch (1936*a*). Even in Harrison's hypothesis discussed in the preceding section molecular orientation serves merely to produce concentration gradients of substance. The hypothesis advanced by Ludwig (1932, pp. 422–27) postulates different *R*- and *L*-agents in opposed concentration gradients as a basis for bilaterality and asymmetry, but how these gradients originate and how they are maintained is not considered. A maximum concentration of one of these agents higher than that of the other determines asymmetry; and if the one of higher concentration is injured, the one that was previously inferior becomes predominant, and reversal of asymmetry results.⁴⁵ The inheritance of asymmetry Ludwig regards as analogous to sex inheritance.⁴⁶ It may be questioned whether the data on symmetry require different *R*- and *L*-agents and opposed gradients. Bilaterality apparently results from a ventrodorsal or dorsiventral differential, and many right-left differences may result from a single quantitative differential between the two sides of the body. If this is the case, resemblance to sex inheritance is less evident.

In all attempts to account for experimental alterations of pattern in

⁴⁵ "Kommt durch Schädigung des prävalierenden das bisher inferiore Agens zum Überwiegen, so tritt eine Umkehr der Asymmetrie ein" (Ludwig, 1932, p. 424.)

⁴⁶ Ludwig, 1932, pp. 427–30; 1935.

terms of concentration gradients it becomes necessary to assume that they undergo change in position and concentration relative to each other in orderly and definite ways; one must "suppress" the other, steepness must change, and concentration must decrease or increase. The assumptions made often seem to be concerned primarily with activity gradients and their changes, though stated in terms of concentration gradients. In any case, metabolism seems to be necessary to bring about the postulated changes in the concentration gradients. If a single metabolic gradient may be associated with two opposed substance gradients, it, rather than the substance gradients, is the effective factor in developmental pattern. Concentration gradients cannot accomplish development without metabolism. Also, differences in concentration of substances in different regions may result from differences in metabolic activity. If we substitute rate or intensity for concentration, many experimental modifications of development present less difficulty to interpretation. In these terms many lateral asymmetries and their reversals appear rather similar to cases of physiological dominance of one side of the body over the other; something of this sort seems to be what Ludwig has in mind when he suggests that injury of the previously predominant agent may result in predominance of the other and so bring about reversal of asymmetry.

At first glance the asymmetries, particularly those with more or less constant laterality, may seem to require an underlying molecular or other spatial structural pattern, genetically determined. But molecular orientation alone cannot determine asymmetry on an organismic scale. As Harrison has recognized, there must be another factor bringing about localization of different developmental potencies on a larger than molecular scale. However, many lateral asymmetries become evident at different developmental stages in different parts. Moreover, they behave experimentally like gradients, not like specific localizations; they show differential susceptibility to external agents, and some of them can be obliterated and reversed in the same ways as other features of axiate pattern. Is it perhaps possible that the genetic factor in these asymmetries consists not in determining an asymmetric structure in the egg but in a definite and constant relation of the developing oöcyte to some inequality or shift in conditions in the parent body determining ventrodorsality or dorsoventrality? Or does presence of polar and ventrodorsal gradient in some way determine pattern in a third dimension? Determination of a third axis or differential by passage of an electric current in a conductor through a magnetic field oriented at right angles to the conductor is cited by Hux-

ley and De Beer as an interesting analogy in a physical system to determination of an asymmetry in relation to other axes already present in organisms. That it is anything more than an analogy they do not maintain.⁴⁷

It is sufficiently evident from preceding chapters that gradients of activity of various kinds—metabolism, rate of cell division, growth, differentiation, etc.—are very generally characteristic of developmental patterns. Whatever our assumptions concerning “intimate structure” of one kind or another, or concentration gradients, the earliest distinguishable and most general form of axiate pattern appears to be a gradient or gradient system on a molar scale, involving the essential activities of the living protoplasm in which it appears. Within the more general systems of earlier stages new, more restricted gradient systems of different kinds arise as activities at different levels of the earlier systems become increasingly different. These activity patterns may bring about definite and persistent patterns of molecular or other structure or orientation, crystalline or micellar. In short, patterns of many kinds may result from the orderly and integrated activities of living protoplasms.

It is evident from earlier chapters that a persistent axiate pattern can originate in a local activation or from a quantitative environmental differential and that at different levels of a primarily quantitative pattern differentiations and new patterns may arise. There is also considerable evidence indicating that ventrodorsality and dorsiventrality are primarily gradient patterns, becoming effective at a later developmental stage than polarity, and after the polar pattern has brought about more or less material alteration along its course. According to this concept, regional differentiations, concentration gradients of substance, specific chemical relations of regions by means of hormones and other substances, and whatever molecular orientation or crystalline structure may be present are all developmental expressions, not primary features of pattern.

The most general and primitive type of pattern on an organismic scale is an excitation-transmission gradient in, or on the surface of, a protoplasm, resulting from transmission with an intensity decrement from a region primarily excited by an external energy. This pattern can arise

⁴⁷ As they point out, when a conductor carrying an electric current and a magnetic field of independent origin are oriented at right angles to each other, the conductor is subjected to a force acting in a direction at right angles both to it and to the magnetic field (Huxley and De Beer, 1934, p. 79). With the magnetic field vertical, the north pole upward, and the conductor carrying current horizontally away from the observer the conductor, tends to be displaced to the left.

without any pre-existing regional differentiation, and it represents the most primitive pattern of organismic behavior in reaction to an external factor. If a region of primary activation persists, the gradient may persist and become a physiological axis, that is, a pattern in which axiate development is possible. Many lines of evidence indicate that axiate organismic pattern in its simplest terms is such a gradient, but it does not follow that individual development always begins with pattern in its simplest terms. Many eggs at the beginning of embryonic development are far beyond this stage.

The gradient pattern represents the "organism as a whole" in its simplest terms. It is the primary and fundamental correlating and integrating factor on an organismic scale. That the organism is more than the sum of its parts is undoubtedly true, for it consists not only of the parts but of the ordering and integrating factors of the gradient system. The unity or wholeness, factor of wholeness, *morphe*, etc., of the organism, is primarily the gradient system and the relation of dominance and subordination resulting from it; this makes possible the spatial and chronological order of development.⁴⁸

Again it must be emphasized that embryonic development constitutes only a small part of the problem of developmental physiology and, so far as beginnings of developmental pattern are concerned, not the most important part. An adequate theory of developmental pattern must include all types of development and must be based on the simpler, not on the most highly specialized, types. There must be a fundamental identity of developmental pattern underlying development of an individual from an isolated piece of another individual, from a bud, from an aggregate of dissociated cells, and from an egg. The concept of developmental pattern as primarily a dynamic gradient system or pattern is an attempt, based on much evidence, to formulate that identity in general physiological terms. Can we conceive all the reconstitutions of wholes from parts—either parts of an egg or embryo or parts of a mature individual—except in terms of an essentially quantitative gradient system, unless we are willing to follow Driesch in postulating a metaphysical ordering and integrating principle, a sort of god in the machine, which we may name "entelechy" or something else, as we please? Driesch argued that an organism is not a "machine," that is, a physicochemical system, because isolated parts of a machine cannot make a whole machine, but isolated parts of many organisms can become wholes. But Driesch was thinking

⁴⁸ Cf. Ritter, 1919; von Bertalanffy, 1932.

of machines with specific localized parts. There are quantitative "machines" which can be divided into parts indefinitely, and each part possesses the characteristics of the whole, only on a smaller scale. A flowing stream, an electric current in a conductor, and, to a considerable extent, a flame are such machines. If axiate organismic pattern is primarily a quantitative gradient pattern, the organism in its simplest terms is a quantitative machine in a specific protoplasm. Parts capable of becoming wholes when isolated differ from the wholes only quantitatively, as far as the factors essential to reconstitution are concerned; if qualitative regional differences are present, they are not essential. As a matter of fact, we know that they are often obliterated. The pattern in an isolated part may differ in scale from that of the whole, according to physiological and external conditions; scale of organization may be larger than the piece, so that a partial axiate pattern results, or smaller than the piece, so that a single axiate pattern does not involve the whole piece. These and many other experimental data are difficult to interpret on any other basis than a primarily quantitative gradient pattern.

Objection to a theory of origin of organismic pattern in relation or reaction to conditions external to the protoplasm concerned has been made on the ground that features of pattern so constant and fundamental as polarity and symmetry cannot be determined by external factors, though they may be modified by such factors. To the theory that metabolism is the essential factor in the establishment of pattern the objection has also been raised that a fixed persistent pattern becoming the basis of morphological development cannot originate in activity, in metabolism, alone. These objections ignore certain important points. First, external factors in their action on living protoplasts only initiate changes; the results depend on the specific constitution and physiological condition of the protoplasm concerned. The physiological condition of the protoplasm is not independent of environment, but within the "normal" range an approach to a steady state is possible. If local action of an external factor determines a local activation and a gradient results, or if an external differential determines a gradient directly, the characteristics of the gradient, its length, its steepness, the reactions occurring in it, and the specific or qualitative differences which develop at its different levels will all depend primarily on the constitution of the protoplasm acted upon, rather than on, the acting agent. The egg of *Fucus* is an excellent illustration. Polarity may be determined in it by differential illumination, by electric current, by a differential in hydrogen-ion concentration, by a stratification

of protoplasmic substances by centrifugal force, and by deformation of the egg (pp. 423-25); but however the polarity is determined, the same sort of individual develops, provided the environment is "normal." In a piece of hydroid stem polarity may be determined by the activation at a cut surface, by electric current, or by an oxygen differential; but the individuals resulting have the same polar pattern, though they may differ in scale of organization.

Second, developmental pattern is constant and uniform for the species only within a certain range of so-called "normal environment," and even within this range uniformity is by no means complete. Alteration in the essential factors of environment beyond a certain relatively narrow range alters developmental pattern in definite and, to a high degree, predictable ways and may even obliterate it. Differential susceptibility of different gradient-levels may bring about alterations so great that without knowledge of the origin of the material its species could not be recognized (see chaps. v-vii). The belief that developmental pattern is highly stable or even autonomous in origin was largely a result of the observational study of embryonic development. With application of experimental methods it became evident that pattern is much less stable and less independent of environment than had been supposed. It is true that the polarity of most animal eggs appears to be relatively stable, but that has been altered experimentally in both invertebrates and vertebrates and even obliterated. Probably if earlier stages of ovarian development of the oöcyte were accessible to experiment, pattern would be found less stable. Embryonic ventrodorsality and dorsiventrality and asymmetry have been experimentally altered, reversed, or obliterated in various species and by various methods.⁴⁹

Third, metabolism, of course, requires a substrate; but a local activation and establishment of a gradient or gradient system may occur without pre-existing regional differentiation in that substrate, and the metabolism may bring about regional differences. Many cases of reconstitution permit little doubt on this point. The effective factor in development appears beyond question to be metabolism; concentration gradients, molecular arrangements, and morphological pattern are apparently results of earlier metabolic patterns, though all of them, when present, may become factors in modifying those patterns.

⁴⁹ See chaps. vi, vii, xii, xiii.

CHAPTER XVII

PHYSIOLOGICAL INTEGRATION, DIFFERENTIATION AND GROWTH IN THE PROGRESS OF DEVELOPMENT

THE course of development is, in general, a progressive physiological and morphological alteration and complication of pattern with appearance of new patterns within those previously present and often with regression and disappearance of certain features of earlier stages, such as larval organs. That development represents the reactions of a protoplasmic or cell system of a certain specific constitution to a spatial pattern seems evident. The primary spatial pattern represents the primary ordering and integrating factor, but the character of the spatial and chronological order in development depends on the specific constitution. This is true not only for the whole organism but for particular organ systems. Spatial pattern permits realization in development of protoplasmic potentialities which cannot be realized in its absence. With approach to a dynamic equilibrium or a steady state which represents the limit of development of which the particular protoplasm is capable in a given environment, development comes virtually to an end.

To deal with details of later development is entirely beyond the present purpose. Only questions of the relations of parts and some of the problems of growth are touched upon. As regards relations of parts, we find, on the one hand, certain ordering or integrating factors, on the other, a capacity for independent differentiation or self-differentiation of certain parts appearing at certain developmental stages in some forms. These two factors are, in some measure, mutually exclusive or antagonistic: ordering and integrating factors represent relations of a part to other parts; self-differentiation of a part represents independence of integrating factors. Growth is an important factor in development for morphological form, and proportions are largely results of differential growth.

INTEGRATING FACTORS

That any form of organismic development is an orderly sequence of events in space and time is evident. Experimental analysis has shown by

reconstitution of isolated parts, by development of parts following transplantation or explantation, by relation of development of various parts to innervation, and by discovery of the role of chemical correlative factors, hormones, etc., that ordering, integrating, or correlating factors play an essential part in development, that a physiological unity is essential to initiation of orderly development.

It was noted in chapter i that two groups of factors are concerned in physiological integration—the transmissive or dynamic, consisting in transmission of energy changes without mass transport of substance from region of origin to region of effect, and the material, transportative, or chemical factors, consisting in production by certain parts of the organism of chemical substances and their mass transport by one means or another and action on other parts. These factors constitute the physiological basis of the unity of the organism, of the organism as a whole.

Transmission of mechanical, thermal, or electrical energies is possible in living protoplasms; but the transmissive factor most important physiologically is transmission of the physiological change known as “excitation.” Difference in electric potential between regions or parts of an organism results from many differences in physiological condition; but the fact that characteristic potential differences are maintained, even when regions concerned are connected by a conductor, indicates that the chief ultimate source of the differences is metabolism. There are grounds for believing that electrical transmission resulting from local activation or from regional differences in activity is the most general and primitive integrating factor on an organismic scale. According to current theory, electric transmission is an essential factor in conduction of the nerve impulse.¹ Granting this, it is doubtless also essential in the more primitive transmissions of excitations or activations in protoplasms generally. The simplest, most primitive sort of organismic or developmental pattern appears to be the spread or transmission, usually or always with a decrement, of the effect of a local activation or excitation of a protoplasm. Such an excitation-transmission gradient is possible without any pre-existing local differentiation of the regions concerned. In consequence of its activation the region of primary excitation or activation becomes, for the time, a dominant region. Various lines of experiment on determination of polarity discussed in earlier chapters show that, if the activation persists, the

¹ See, e.g., R. S. Lillie, 1922, 1923, 1936; Adrian, 1932; and citations by these authors. The action currents associated with nervous conduction have been studied by a host of investigators for many years.

protoplasmic substrate within the range of the transmitted effect may be so altered that a persistent gradient may be established and become an axiate developmental pattern.

The early differentiation of the central nervous system and the general localization of the chief aggregations of nervous tissue at higher levels, not only of the polar but of the ventrodorsal or dorsiventral gradient, suggest that it represents the most direct physiological and morphological expression in later development of the dynamic factors of earlier stages. Moreover, it evidently represents the highest development in each species-protoplasm of the dynamic integrating factors. This remains true for the vertebrates, even though an inductor is apparently necessary for neural development. The region known as the "neural inductor" in amphibian development is a secondary feature of developmental pattern; and direction of its invagination is evidently not independent of the primary pattern, nor is the localization of anterior regions of the central nervous system determined solely by the inductor in development under natural conditions. And finally, it is not yet demonstrated that the natural inductor is a substance; but whether it is or not, the presumptive chorda-mesoderm is itself an expression of earlier developmental pattern rather than the basis of pattern.

The relation to the general gradient pattern of localization of the central nervous system and of nervous dominance does not exclude the possibility that various factors, specific chemical, electrical, or mechanical, may be concerned in determining distribution and direction of growth of axons. The suggestion that axons grow up the gradients, dendrites down,² does not seem entirely in accord with the facts. Certainly in the vertebrate embryo the earlier axons appear to grow down the gradients; and in the final pattern of the spinal cord the long axons are those which grow down, while the afferent, upward-growing axons are, in general, short, as if able to grow only a short distance against some opposing factor. In general, the chief aggregations of nervous tissue are at higher gradient-levels, and the nerves grow down. Local conditions and their changes during development are doubtless concerned in determining the progressive increase in complexity of directions of growth of axons, particularly within the central nervous system. It has been maintained, on the basis of tissue culture experiments, that a physical ultra-structure determines the course of growth of nerve fibers (Weiss, 1934). Whether such structure is the only directive factor is still an open question.

² Huxley and De Beer, 1934, p. 380.

The integrating action of the nervous system in the functional life of higher animals is sufficiently evident from a great variety of experiment and the resulting literature and also from daily life. Moreover, although nervous function is not essential for differentiation of certain parts—for example, vertebrate striated muscle—it is necessary for maintenance of muscle structure after differentiation. Apparently the level of metabolism in the developing muscle is high enough for its differentiation without nervous stimuli, but in the differentiated muscle the intrinsic level sooner or later falls below that necessary for maintenance of its structure. Reconstitution and grafting experiments suggest that in planarians, probably in hydroids, and perhaps in annelids, the nervous system is the chief integrating factor in adult life.

The fact that nervous stimuli do not appear to be important or essential as integrating factors during most of embryonic development may appear, at first glance, to conflict with the view just advanced. Actually, however, nervous function is merely the highest development of a function which we believe to be common to all living protoplasms, that of excitability. Even though transmission of excitation by differentiated nerves plays little or no part in embryonic and some other forms of development, more primitive transmissions of effects of activations or excitations undoubtedly do occur and play an essential part in early physiological integration. After the general pattern of an organ system is established, it may become temporarily more or less independent of pattern in other parts and may undergo more or less self-differentiation, but it is later integrated into the whole, at least in part by nervous factors.

Production of a specific substance by one part of an organism and its mass transportation to, and action upon, another part is obviously possible only when some degree of difference is present in the parts concerned. As long as they remain qualitatively alike, they produce the same substances, though perhaps at different rates. Differences in concentration of metabolic products at different gradient-levels, resulting from different rates of production, may be concerned in determining further differences; but such effects do not constitute specific chemical relations between parts. Theoretically the specific chemical effect of a product of one part on another becomes possible as soon as specific differentiation of the parts and production of different substances begins. Apparently, however, it does not play a very important part in integration until a considerable degree of differentiation has been attained. The work of recent years in endocrinology and on hormones and other chemical products of metabo-

lism has demonstrated their importance in both animals and plants and has also shown that their effects and interrelations become increasingly definite and complex with increasing differentiation and are therefore most conspicuous and most essential in the higher animals and man. Nevertheless, since these specific chemical relations are possible only after the parts concerned have become at least chemically, and perhaps morphologically, different, they cannot initiate differentiation or developmental pattern; they are secondary, not primary, factors in development—expressions of pattern already present. They may, of course, play important parts in influencing the further course of development and the character of function.

INTERRELATIONS OF FACTORS IN INTEGRATION

Actual physiological control and integration may result from the combined action of dynamic and material factors, and the interrelations of the two groups become exceedingly complex and varied in the higher vertebrates and man. It is now a familiar fact that in these organisms hormones may affect the nervous system and alter nervous reactions and general behavior in many ways. Effects of the sex hormones, adrenalin, etc., on behavior are cases in point. And it is no less true that nervous stimuli may influence hormone production or liberation, adrenalin again being an example. The chemical mediation of nerve impulses by formation or liberation at nerve endings of acetylcholine or sympathin represents a still more intimate relation of dynamic and material factors.³ Possibly it is not without significance in this connection that the vertebrate hypophysis, which appears to exercise, in some measure, a dominance in endocrine relations, is localized, like the chief aggregations of central nervous tissue, in the high region of the polar gradient.

These interrelations, however, have to do with the mature life of higher animals and undoubtedly originate late in development. We have less information concerning interrelations of dynamic and chemical factors in earlier developmental stages, though they may exist after differentiation has taken place. It is reported that presence of the larval cephalic nervous system is necessary in certain insects for metamorphosis from larva to pupa, supposedly because of a substance, a hormone, produced by it.⁴ The phytohormone, auxin, is generally produced in larger amount in the apical region than elsewhere in the plant axis and is transported more

³ W. B. Cannon and Rosenblueth, 1937; W. B. Cannon, 1939.

⁴ Kopeč, 1922; Kühn und Piepho, 1938; Plagge, 1938.

readily basipetally than acropetally. The axial differential in production and the directional differential in transport, whether electrical, as at one time suggested, or dependent on some other factor, indicate relation to gradient pattern.

Interrelations within the group of dynamic factors attain a delicacy and complexity far beyond our present understanding in the functional activities of the central nervous system. The variety and complexity of interrelation within the chemical group, particularly in the higher vertebrates, are coming to light with the progress of investigation of hormone action.

It is evident that the specificity and complexity of integrative interrelations increase during development. Transplantation experiments show that, in general, tissue-specificities, individual-specificities, and even species-specificities are less evident in earlier than in later stages. But whatever the conditions in later stages, it is evident that an orderly organismic development to an integrated whole is not possible without an underlying general ordering and integrating pattern of some sort—not merely a structural substrate but a physiologically effective activity pattern.

INDEPENDENT DIFFERENTIATION

In contrast to the ordering and integrating features of developmental pattern and the dominance and subordination associated with it, experimental isolations and transplantations of parts show that at certain developmental stages some parts become capable of continuing differentiation for a time in other than the normal organismic environment or in complete isolation from the rest of the organism. Such parts are said to be determined and to undergo self-differentiation or independent differentiation, as distinguished from dependent or correlative differentiation. Also, removal of certain parts at certain stages results in permanent defects, other parts being unable to reconstitute them. As far as parts show this capacity for self-differentiation, development, or the stage in which the capacity is present, has been regarded by many as a mosaic of independent parts. Huxley and De Beer (1934) head their chapter vii: "The Mosaic Stage of Differentiation," and give many examples of independent differentiation, in large part from amphibian development. Many interesting questions center about this capacity for self-differentiation. All our knowledge of the progress of determination and differentiation and their stability or irreversibility results from experiments for the purpose

of discovering whether, or to what extent, this capacity is present in particular primordia at particular stages. We know that in vertebrate development different primordia attain the condition permitting independent differentiation at very different stages of development of the embryo—some earlier, some later. Moreover, a part or organ system may be dependent in certain respects, independent in others; an amphibian limb bud, for example, is determined as a limb before its dorsiventrality is fixed (pp. 285–88). The concept of mosaic development has in most, if not in all, cases only relative significance. It was shown in chapter xiv that even in organisms with determinate cleavage development is far from being the complete mosaic that it was formerly supposed by some to be. Even in amphibian development there is no single stage of development of the whole organism at which it becomes a mosaic of independent parts. Moreover, even though an optic primordium or a limb bud becomes capable at a certain stage of more or less self-differentiation, the differentiation of parts of these primordia is not independent of other parts and in the urodele limb never becomes so.

Capacity for independent differentiation does not necessarily involve any visible differentiation; but in complex organ primordia, such as the limb bud or the eye, a definite developmental pattern must be present and must persist unaltered after isolation from the normal organismic environment. Considering, again, the amphibian limb primordium, it undoubtedly has become different in some way from other regions at a certain stage, whether we call this difference “chemodifferentiation,” with Huxley and De Beer, or “invisible differentiation” (Gilchrist, 1937) or merely “determination.” But for development as a limb, not only this difference but an axiate pattern is necessary. Histological differentiation of a single cell may result from chemodifferentiation of the cell, but for orderly differentiation of an organ system independently of other parts a developmental pattern is obviously necessary. In the limb primordium this is apparently primarily a gradient pattern in the chemodifferentiated, invisibly differentiated, or determined limb region.

Independent differentiation of transplanted or isolated parts is further illustrated by many other data discussed in earlier chapters. Amphibian notochord, otic, branchial, and various other primordia acquire more or less capacity for independent differentiation at a certain stage of their development but at different stages of the embryo. Chorio-allantoic grafts of portions of chick blastoderms show considerable differentiation in the altered environment, but many of them give rise to more tissues or organs

than in their normal environment; that is, more or less reconstitution occurs, indicating that their differentiation is not wholly independent (pp. 529-35). The insect embryo is not a mosaic in early stages but, at least as regards ability of larger regions to continue differentiation independently for a time, approaches mosaic condition rather early, though integrating factors are essential in metamorphosis.

According to the earlier predeterministic theories of development, every differentiation is essentially a self-differentiation; but experiment has made it evident that this is far from true. At present the capacity for self-differentiation of a part is generally regarded as a condition appearing in the course of development, earlier in some forms and in some parts than in others. Weiss has called it an "autonomization" (1926a) or "emancipation" of parts from the whole (1935, 1939). The emancipation is rarely, if ever, complete. Transplanted or isolated self-differentiating parts very commonly show some departure from the normal; they may show some reconstitution, developing more than they would normally; they may develop less than normal; their development may continue for only a short time, and differentiation is often less complete than normally; even if histological differentiation is normal, departure from normal morphology is frequent. Moreover, whatever the degree of emancipation at a certain stage, it is not permanent.

Perhaps of even greater significance for an adequate conception of development than occurrence of independent differentiation is its absence, except in the dominant region, in some of the simpler animals. Reconstitution of wholes from isolated blastomeres, parts of blastulae and of planulae, and also from pieces of the mature individual proximal to the hydranth takes place in many coelenterates. The hydranth of *Tubularia* or *Corymorpha* and of various other forms, or even its extreme apical region, can differentiate quite independently of other parts; but no other region of the hydroid body is capable of independent differentiation at any stage of life. Medusa buds and probably some gonophores, after attaining a certain stage, are capable of more or less independent differentiation; but they, like the hydranth, are dominant regions. As regards potentialities of parts of the planarian embryo, nothing is known; but in postembryonic reconstitution the head can develop quite independently of other parts, but no other part of the body is capable of independent differentiation. A hydranth or part of a hydranth or a planarian head, when isolated, does not give rise to other parts; but other parts reconstitute hydranth or head, and these induce development of other parts.

Annelid reconstitution shows essentially similar conditions. In all these forms only the dominant region at the high end of the polar gradient is capable of independent differentiation, and every body-level will develop as dominant region unless it is subordinated to a dominant region or inhibited by external conditions (chaps. ix-xi).

Within the hydranth primordium there is apicobasal dominance. Removal of the apical region during hydranth reconstitution may result either in regression of the remaining basal portion and reconstitution of a new hydranth or in regeneration of the apical region, according to stage and level of removal. In the planarian head the cephalic ganglia evidently constitute the dominant region of the mature individual, and this dominance is essential to persistence of the individual. The differentiation of explanted planarian parenchyma into cells and fibers identical in appearance with explanted ganglion cells and their outgrowths (Murray, 1927, 1931) suggests that these ganglia are the self-differentiating regions of the head in normal development. Even if this is true, however, the independence is probably not absolute, for in grafts of the ganglionic region into the ganglionic region of a host polarity of the graft may be altered or reversed (p. 382). The mosaic characteristics of annelid and mollusk development, as far as determined, concern larval parts which represent early differentiations of anterior regions. There is considerable evidence that the presumptive trunk region, at least in annelids, is not a mosaic (p. 558). Even in these forms the difference between embryonic and adult stages, as regards independent differentiation, is perhaps not as great as has been believed. In short, in hydroids and planarians and apparently in many annelids, subordinate parts are never emancipated; their persistence as subordinate parts always remains dependent on dominance of higher levels. Emancipation of parts or attainment of mosaic condition is by no means a universal characteristic of development. Completely dominant regions do not become emancipated but are independent from their initiation. Other parts may, in some animals, attain capacity for independent differentiation, either when they have undergone a certain degree of determination or differentiation in relation to other parts or when a local developmental pattern involving dominance and subordination has been established in them. Ability of a part to continue differentiation for a time independently of its normal relations to other parts does not necessarily prove that it is independent in the intact animal. Self-differentiating parts may be accomplishing more in the way of development than normally. Whether there is complete isolation or emancipa-

tion of any other parts in the intact animal than those completely dominant from the beginning, such as the hydranth region of a hydroid, may at least be questioned.

GROWTH: THE QUESTION OF DEFINITION

In any consideration of the many problems involved in the changes that biologists have called "growth" in living organisms, the question of definition must be raised. Many definitions of "growth" have been given, and the term has often been used without definition for various features of development. There is still no general agreement as to what constitutes growth. For some biologists growth is increase in size or weight. Such increase may occur in many ways—by increase of living protoplasm; by increase of fat, or in plants of starch; by swelling of protoplasm or other cell constituents in consequence of absorption of water; by increase in size of cell vacuoles; and by deposition of products of metabolism—chitin, keratin, cellulose, mineral salts in various structural forms, skeletons, shells, etc. Some of the plant physiologists have maintained that growth involves change of form. For example, Sachs (1887) defined growth as increase in volume intimately associated with change of form. According to Pfeffer (1901), growth is permanent change of form in the protoplasmic body, and increases in volume or mass are not correct criteria of growth. Thompson (1917, p. 52) says: "The transference of portions of matter into the system from without and from one widely distant part of the organism to another" is what is usually regarded as growth. He points out, further, that on the basis of this concept of growth the modifications of form in organisms depend essentially on difference in rates of growth, in different directions, except in so far as purely molecular forces are concerned. In his study of relative growth Huxley (1932, p. 6) says: "One essential fact about growth is that it is a process of self-multiplication of living substance." Elsewhere (p. 149), however, he distinguishes the "multiplicative, intussusceptive or compound interest type of growth," in which the increment of material is alive and contributes to further growth and "the additive, accretionary or simple interest type," in which the increment consists of nonliving material that makes no further contribution to growth. It is obvious that the additions of different kinds of materials take place under different conditions and involve different activities. If growth includes all additions, the physiology of growth presents not one but many problems.

There is still another aspect of protoplasmic activity involving change

in volume or weight, to which comparatively little attention has been given in studies of growth: that is, negative growth or reduction. Growth as increase in size or weight is not irreversible. In the absence or inadequacy of nutritive material or other substances contributing to increase, or under physiological or pathological conditions that increase catabolism, reduction in size and in weight of the organism may occur, and this may also be differential as regards parts of the body. In the warm-blooded animals reduction soon results in death, but cold-blooded vertebrates can undergo much greater reduction; and some of the lower invertebrates—for example, planarians—can undergo reduction by starvation to a minute fraction of the original size and weight and still remain active and in good condition, and even capable of reconstitution. When feeding is resumed, positive growth begins, and the original size may be again attained. In planarian starvation the digestive tract undergoes reduction more rapidly than other organs, with progressive disappearance of its branches from their tips basipetally. In absence of digestive function this organ serves, to a greater extent than any other, as a source of nutrition. Even in vertebrates absence of function in a differentiated organ—muscle, for example—results in its reduction, often in its complete disappearance.

Perhaps, then, growth in living organisms should be regarded as including not merely increment but both increment and decrement of substance, as transfer of substance either to or from an organismic system as a consequence of protoplasmic constitution and activity.

GROWTH AND DEVELOPMENT

The relation of growth to development is by no means uniform, either spatially or chronologically. An organism may undergo extensive development without growth increment of the whole, or even during reduction, as in planarian reconstitution during starvation and in early stages of embryonic development of most animals. Certain regions of the reconstituting individual grow at the expense of others, and in the embryo protoplasm grows at the expense of nutritive material; but the total result in both cases is reduction. The histological differentiation of cells and growth, associated with cell division and synthesis of protoplasm, appear to be more or less mutually exclusive. When cells undergo visible histological differentiation, they usually cease to divide and very commonly cease to grow or grow but little or in an entirely different way from the dividing cell, perhaps by swelling or by increase in size of vacuole, as in

many plant cells. If, for any reason, they resume division, they lose their visible differentiation, though not necessarily their specificity; and the earlier type of growth by synthesis of protoplasm may again appear. In regeneration of striated muscle, for example, cytoplasm without visible differentiation accumulates about the muscle nuclei, and spherical dividing cells with protoplasmic growth result; later they cease dividing and differentiate into muscle. Many other similar cases appear in reconstitutions.

In all except spherical organisms growth differs in rate and amount in different directions and is the chief factor in determining the specific forms of organisms and their parts. Different kinds of growth may occur in different parts: some may grow by synthesis of protoplasm, others by swelling, deposition of skeletal material, etc. Changes in rate and character of growth may also occur in a region or organ in the course of development. In general, growth rate decreases with progress of development, at least after early embryonic stages; but the decrease may be modified by growth cycles, by appearance of new growth centers, by metamorphosis, and by other factors. Differential growth of certain parts, the hydroid tentacle, the sea-urchin archenteron, and the amphibian optic primordium, becomes evident at certain developmental stages; that of others, hydroid stolons, arms of the sea-urchin pluteus, and the amphibian limb, at other stages. In regenerative reconstitution growth rate of regenerating parts is usually much above that of others. In starving planarians growth of the regenerating head and posterior end accelerates reduction of the whole. Growth patterns of reconstitution by reorganization without regeneration, as in *Tubularia* and in *Corymorpha*, do not show these extreme differences in growth but approach more nearly embryonic patterns. Growth of protoplasm is very generally characteristic of earlier embryonic stages and earlier stages of particular organs; as development progresses, other kinds of growth appear. The plant axis provides an interesting example of both spatial and chronological differences in growth. Growth by increase of protoplasm is characteristic of earlier embryonic and bud stages and persists in the vegetative tip throughout its growing life; but as cells become farther removed from the tip by its continued growth, protoplasmic growth decreases or ceases, vacuolization begins, and most of the increase in size of the plant results from enlargement of the vacuole, from formation of substances that swell by uptake of water, from formation of cellulose, and from deposition in storage organs of starch. But whatever the changes in rate and kind of

growth during development, spatial and chronological growth patterns are orderly and definite in axiate organisms.

Most, perhaps all, growth patterns can be altered experimentally in one way or another: some are altered by autoplasmic, homoplasmic, or heteroplasmic transplantation to other regions, though some growth patterns tend to persist under these conditions. Changes in external environment of the whole organism are highly effective in altering growth pattern, as the differential modifications of development show (chaps. v-vii). Alterations in nutrition, mineral salts, etc., may also be effective. Growth patterns are evidently related in some way to the gradient patterns of development, but the relation is not necessarily direct and simple.

A characteristic of malignant growths, and apparently an essential factor in malignancy, is lack of a definite organismic pattern of growth and differentiation. More or less differentiation of cells, according to the organ of origin of the neoplasm may take place, but orderly pattern on a multicellular organismic scale does not appear.

SPECIES-SPECIFIC GROWTH RATES AND SIZE FACTORS

Transplantation experiments, particularly those with amphibian embryonic materials, have brought to light certain interesting and significant facts concerning growth rate and size of certain organs in organismic environment of another species, as a few examples will show. When sufficiently fed, *Amblystoma tigrinum* grows much more rapidly than *A. punctatum* and attains about double the latter's size. With feeding up to the maximum intake, optic and limb primordia transplanted from one to the other species show, during most of the larval development, the same growth rate as the normal organs on the donor species and, consequently, become very different in size from the host organs. Before metamorphosis, however, the eye and the limb of *A. punctatum* on *A. tigrinum* become slightly larger than the normal organs of *A. punctatum*.⁵ In earlier experiments it had been found that under ordinary nutritive conditions *A. tigrinum* eyes and limbs on *A. punctatum* hosts grew not only more rapidly than the host organs but more rapidly than those of the donor-species control (Harrison, 1924; 1929a, b). With *A. tigrinum* as host, growth of *A. punctatum* organs is retarded. The work of Twitty and Schwind showed that these alterations in growth rate were due, at least in large part, to differences in "nutritive level," that is, to underfeeding of the *A. tigrinum* larvae which have the higher nutritive requirement. Twitty and Schwind

⁵ Twitty and Schwind, 1928, 1931; Schwind, 1931.

suggested that the experiments indicate a similar organismic environment regulating growth rate in the two species, rather than fixity of the intrinsic rate. With this possibility in mind, Twitty made heteroplastic transplantations of optic primordia, using individuals of the same two species but of different age or growth stage and found that growth of the eye, relative to that of the host, could be retarded or accelerated. With older *A. punctatum* as host, younger eyes of *A. tigrinum* grow very rapidly, while growth of host eye and normal eye is prevented by light feeding. With younger *A. tigrinum* as host, growth of transplanted eye is inhibited, even with liberal feeding and rapid growth of host. "Expression of the growth capacity of the eye is primarily a function of the relation between the physiological conditions in the organ and in the environment provided by the host, rather than of mere growth in size of the latter" (Twitty, 1930).

When the optic vesicle alone, without lens or corneal ectoderm, is transplanted from one species to the other, the developing lens apparently influences growth of the transplanted eye. The optic vesicle of *A. tigrinum* transplanted to *A. punctatum* grows more rapidly than the eye of the host species but more slowly than an *A. tigrinum* transplant of vesicle with lens ectoderm. This retardation is attributed to the host lens, which is "too small" for the transplanted optic cup. Conversely, the *A. punctatum* vesicle without lens ectoderm, transplanted to *A. tigrinum*, grows more slowly than that of the normal host but is accelerated by the *A. tigrinum* lens, which is "too large." Transplants of lens ectoderm alone from *A. tigrinum* to *A. punctatum* give rise to lenses too large for the eye. Their growth is retarded, but growth of the host optic bulb is increased. In reciprocal transplants of lens ectoderm the lenses are too small, but their growth is accelerated and that of the bulb retarded.⁶ Evidently the species-specific growth rates of these parts of the eye, which normally develop in a definite relation to each other, may be mutually altered when the parts originate from different species. This seems not to be true for all parts of the embryo, for heteroplastic transplants of portions of the shoulder girdle between the same two species do not influence growth of host parts but may themselves be altered in form. On the other hand, transplants of the larger limb primordium of *A. tigrinum* to *A. punctatum*

⁶ Harrison, 1929a, b; Rotmann, 1939, in experiments with other species. It has also been shown that heteroplastic eye transplants between the same two species influence growth of various parts of the brain, of the cartilaginous capsule developing about the eye, and of the eye muscles (Twitty, 1932).

which become articulated to the shoulder girdle of the host induce hypertrophy of the latter. Also, the *A. tigrinum* limb on *A. punctatum* usually induces hyperplasia, and the reciprocal transplantation, hypoplasia of the spinal ganglia, innervating the limb (Schwind, 1931, 1932). Heteroplastic grafts of heart primordia between the same two species give hearts functionally donor but with growth rate altered toward that of the host (Copenhaver, 1939). *Amblystoma tigrinum* somites grafted in place of *A. punctatum* somites become much larger than those of the host (Detwiler, 1938).

According to these data, species-specific growth rates and amounts show more or less tendency to persist with heteroplastic grafting but can be altered by relations to the host. That different basal metabolic rates of different species and stages may be factors in the experimental results seems not improbable. The "nutritive level" may be largely metabolic level. *Amblystoma tigrinum* eyes and limbs on *A. punctatum* under ordinary nutritive conditions grow even more rapidly than in the donor species, perhaps because with the low metabolism of the host under submaximal feeding they are able, in consequence of their high metabolism, to obtain even a larger relative amount of available nutrition than under normal conditions. Older *A. punctatum* eyes transplanted to younger *A. tigrinum* are retarded, perhaps because of their low metabolism, compared with that of the host.

Final size of the individual and of its parts is dependent not only on intrinsic growth rate but on initial size and length of growth period, and also, of course, on amount and adequacy of nutritive material available and on other environmental conditions. Primordia of various organs, eye, limb, balancer, etc., in *A. tigrinum* are initially formed on a larger scale than those of *A. punctatum*. In "normal" environment most animal species attain more or less definite and characteristic size and proportion because both intrinsic genetic factors and environmental conditions do not differ greatly for different individuals. With alteration in organismic or external environment, size and proportions may be altered, as is evident from the above experiments and from the differential modifications of development discussed in chapters v-vii.

GROWTH GRADIENTS

Gradients in rate or amount of growth are very generally characteristic of all stages of development. In early stages growth is generally not spatially localized by sharply defined boundaries but decreases radially,

asymmetrically, or axially from regions of highest rate of growth, the growth centers. Adventitious buds of plants and buds in many animals are, in their earliest stages, radial growth-gradient systems (see Figs. 1-4) and become axial, heteropolar gradient systems in consequence of differential growth. Many organs—tentacles of most coelenterates, bryozoa, and other forms; arthropod appendages; vertebrate limbs; etc.—begin their growth as budlike systems, but many of them are not strictly radial. The hydra bud shows a differential in tentacle development, apparently in relation to the longitudinal gradient of the parent body (pp. 634-35). In the amphibian limb an anteroposterior, and later a dorsiventral, differential, both influencing later growth, are determined in relation to the general body axes; but the longitudinal axis of the limb is the result of differential growth in the asymmetrically radial primordium. Even if there is no growth of the whole in early embryonic development, for example, during cleavage, there is usually more or less differential growth, the regions about the apical pole growing more rapidly than, or at the expense of, other regions. The growth-gradient systems of early stages usually, if not always, coincide with the gradient systems otherwise indicated or demonstrated. Primordia of organ systems and organs usually become evident to the eye as growth-gradient systems. Various organ primordia of the chick embryo in very early stages are characterized not only by growth-gradient systems but by dye-reduction gradients in low oxygen after staining, the two being coincident, as far as can be determined. The growth-gradient system of the anal arm of the sea-urchin *pluteus* can be completely obliterated by inhibiting agents; and when it is so obliterated, neither dye-reduction nor susceptibility gradients appear.

In the course of development the boundaries of organs and organ systems usually become more definite, and growth of these organs and systems is correspondingly limited; but within those limits growth gradients may still be present, and the body as a whole may also show growth gradients in these later stages. These are by no means always simple and often change in form with time. Data and graphs for many such gradients have been given by Huxley (1932, chap. iii). In some crustacean appendages there is a decrease in both directions from a growth center at a certain level. Also, the steepness of the gradients and the position of the center may change, and new centers may appear in some of these appendages. As development progresses, the growth gradients become increasingly species-specific in their spatial characteristics, though they may

differ in certain organs of the two sexes, male and female chelae of certain decapods, for example, or even on the two sides of the individual body, in decapods with unequal chelae. Although the growth gradients of later stages may differ widely from the gradient patterns of early development, it appears, beyond question, that they are in some way related to, and developmental consequences of, the earlier pattern and the specific constitution of the protoplasm in which it appears.

SOME OTHER ASPECTS OF GROWTH PROBLEMS

Certain inorganic systems which show a sort of growth and development have often been compared with growth and form in organisms. The analogy of crystal growth and reconstitution of form to organismic growth has been pointed out repeatedly, and a crystalline structural pattern has been postulated by some biologists as the basis of developmental pattern.⁷ Various physicochemical experiments have been devised in which diffusion and precipitation of inorganic substances in a colloid substrate result in growth, often definitely directed, of a precipitate and development of form. Inorganic "cells" have been made, and various simulations or models of cell form have been produced, with inorganic substances. The Liesegang rings, patterns resulting from diffusion and precipitation of inorganic salts in a gel substrate under certain conditions, have been regarded by some as highly significant in relation to organismic form. Under certain conditions surface tension may be a factor in producing definite form. Tension and pressure influence growth and are essential factors in determining form of various parts of organisms. The book *Growth and Form* by D'Arcy Thompson (1917) presents a most interesting and valuable discussion of these data and of the physical characteristics and problems of growth.⁸

Mathematical analyses of various aspects of growth and form in organisms have produced many interesting results which cannot be discussed here. Certain of them, particularly those having to do with organismic form, are presented in Thompson's book. A recent analysis of differential or relative growth, that is, of growth rates of parts or organs in relation to rate of the whole organism, formulates a law of constant differential

⁷ See pp. 5, 296, 394, 629, 694.

⁸ See also Liesegang, 1907, and other papers for periodic patterns resulting from diffusion of salts in a colloid substrate, and the summary by Zeiger, 1939, of papers concerned with patterns of this type. Various inorganic growth patterns and simulations of organismic growth and form are described by Leduc (1910).

growth ratio. This law states that the ratio of growth rate of certain parts to growth rate of the whole body is constant for considerable periods.⁹ The data on which the law is based concern growth in stages following histological differentiation and are chiefly from arthropods and vertebrates; but in these stages the law has been found, according to Huxley (chap. i), to hold for a considerable number of organs of both animals and plants. This growth of parts at a different rate from the whole is heterogonic or allometric growth.¹⁰ It results in a continuous change of proportion of the part to the whole, which may be either positive or negative. However general the validity of the law may prove to be for growth ratios of parts to wholes in later developmental stages, the question of its significance for many growth patterns of embryonic stages remains open.

GRADIENT PATTERNS AND EVOLUTION OF MORPHOLOGICAL FORM

Quite apart from the question whether a gradient pattern is the primary pattern of development, it is evident that evolution of morphological form is, to a considerable degree, a matter of change in gradient pattern. Although the characteristics of a gradient pattern depend on the constitution of the protoplasm in which it appears, not on the initiating factor, it and the resulting development can be altered experimentally in a single species-protoplasm by altering physiological condition. The differential modifications of development are cases in point (chaps. v-vii). How extreme such modifications may be is indicated by the differential modifications of echinoderm embryonic development (chap. vi). Alteration of form and proportions of the pluteus, exogastrulation, and decrease or obliteration of differences along polar and ventrodorsal axes by differential inhibition, changes in form and proportion in the opposite direction by the secondary modifications of differential tolerance, conditioning, or recovery—all these in their various degrees result from differential physiological effects of external agents on gradient patterns. Many of these modifications are highly suggestive as regards evolution of echinoderm larval form. For example, certain degrees of differential inhibition of asteroid

⁹ The law is expressed by the equation $y = bx^k$, in which x is the magnitude of the animal, as determined by standard linear measurement or by its weight minus the weight of the organ concerned, y is the magnitude of the differentially growing organ, and b and k are constants (Huxley, 1932).

¹⁰ Originally called "heterogonic" by Huxley, 1932; the term "allometric" was suggested by Huxley and Teissier, 1936.

development with an agent having high differential action, such as LiCl, give forms resembling sea-urchin gastrulae. With less extreme differential action starfish larvae approaching crinoid larvae in form can be produced. With secondary modification of gradient pattern by tolerance, conditioning, or recovery, echinid larvae showing some approach to the asteroid larval form result. These experimental modifications suggest that evolution of echinoderm larval form has resulted in part from changes in gradient pattern in consequence of change in specific constitution of the protoplasm.

The various head forms resulting from mediolateral differential inhibition in planarian reconstitution, constituting a continuous series from normal to acephalic, with obliteration of parts progressing laterally from the median region (pp. 177-96), and the essentially similar results of differential inhibition in vertebrate development (chap. vii) provide further illustrations of the dependence of forms and proportions on physiological condition of the protoplasm. The experimental alterations of scale of organization (chap. x) show another aspect of this dependence. In all these and many other developmental modifications the gradient pattern has been altered differentially, with resulting alterations in rates and amounts of growth and in localization, degree, or even presence or absence of certain differentiations. Since these differential modifications of growth and form appear to be results of primarily quantitative differential alterations of gradient pattern, they suggest the possibility that at least some of the characteristic differences of growth and form in related species may also be results of similar alterations, primarily quantitative, rather than specific in character, so far as gradient patterns are concerned. Length and steepness of gradients, rates and amounts of growth, and localizations of new growth centers may be altered by genetic changes in protoplasmic constitution, as well as by environmental conditions.

By the use of Cartesian co-ordinates Thompson (1917, chap. xvii) has made evident graphically some of the changes in distribution of growth in the bodies and in various parts of related species. The procedure consists in applying a network or grid of rectangular co-ordinates to the outline of the body or part concerned in one species and deforming or transforming the co-ordinate grid in a regular manner so that it becomes or approximates a corresponding co-ordinate system for the body or part to be compared with the first. The deformation or transformation required in the second case indicates, in a general way, when compared with the first, the differences in growth distribution between the two in certain

dimensions. The method can, of course, also be used for different developmental stages of the same species. Values and limitations of the method are discussed by Huxley (1932, chap. iv).

A few of Thompson's figures are reproduced here. In Figure 222 the method is applied to carapaces of several genera of crabs. In Figure 223

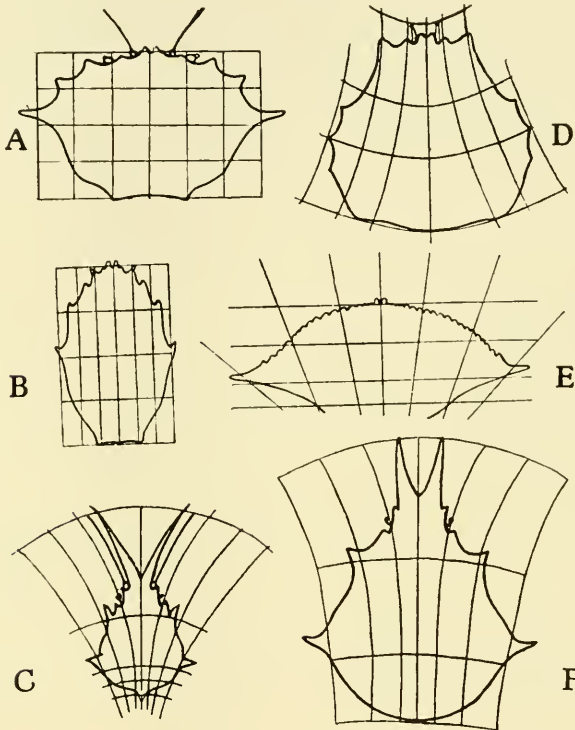


FIG. 222, A-F.—Transformations of Cartesian co-ordinates applied to carapaces of different genera of crabs. A, *Geryon*; B, *Coristes*; C, *Scyramathia*; D, *Paralomis*; E, *Lupa*; F, *Chorinus* (from Thompson, *Growth and Form*, 1917).

it is applied to the whole bodies, viewed laterally, of two related teleosts. In this case transformation of the rectangular co-ordinates, applied in Figure 223, A, to *Diodon*, into a system approximately hyperbolic and transferring the outline of A to corresponding points in it gives, in Figure 223, B, essentially the outline of *Orthogoriscus*, a genus closely related to *Diodon* but very different in form. Evidently, differential growth is much greater in the dorsiventral dimension of the posterior region in *Orthogoriscus* than in *Diodon*. In Figure 224 the transformations required

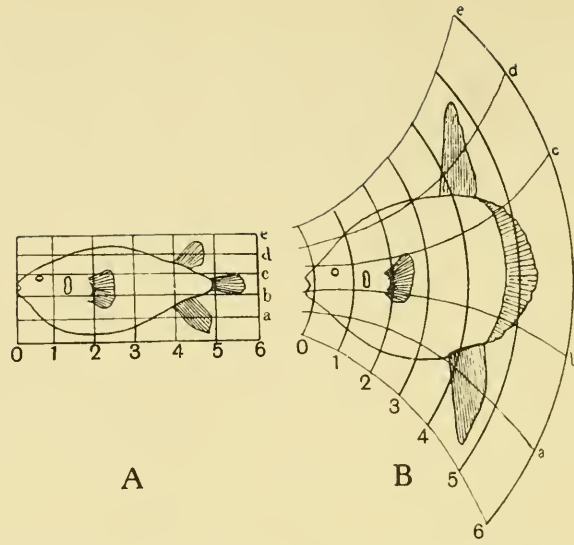


FIG. 223, *A, B*.—Transformation of Cartesian co-ordinates from *Diodon*, *A*, to the closely related *Orthogoriscus*, *B* (from Thompson, *Growth and Form*, 1917).

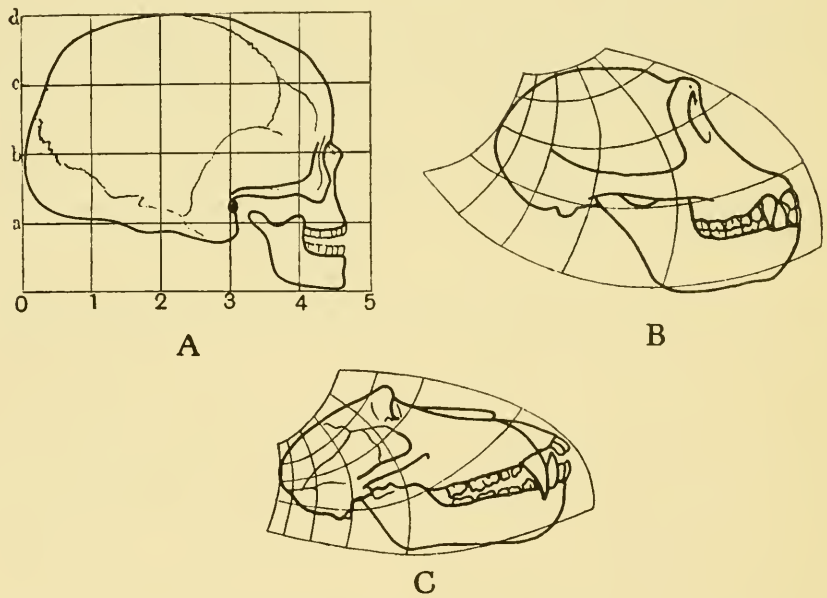


FIG. 224, *A-C*.—Transformations from human skull, *A*, to skull of chimpanzee, *B*, and baboon, *C* (from Thompson, *Growth and Form*, 1917).

for the skull of the chimpanzee (*B*) and the baboon (*C*), as compared with the human skull (*A*), are shown. The transformations in *B* and *C* are of the same order, differing only in degree. That alterations of gradient patterns are concerned in the differences in form indicated in the figures seems evident.

If gradient characteristics, length, steepness, etc., are concerned in determining localization of particular differentiations, it is evident that genetically determined changes in these characteristics may alter localizations of organ systems and organs relatively to others. The kind of differentiation that takes place at any particular gradient-level may be altered by other genetically determined changes in constitution of the protoplasmic substrate. If gradient patterns are essential factors of development, it is to be expected that genetic changes altering gradient patterns may often involve features apparently only slightly related or quite unrelated. The genetic change may alter whole gradient systems, either the general systems of the whole body or the systems of particular organs, and so alter localization, differential growth, and differentiation of many parts. The possibility that a single mutation may determine many such alterations can scarcely be denied. In so far as genetic changes permitting survival and reproduction have effects of this sort on the more general gradient patterns, organisms or organ systems evolve more or less as wholes with orderly relations of their parts.

APPENDIX I

Some further consideration of methods of determining respiration, as used in attempts to discover whether regional respiratory differences are present along the polar axis and of objections and criticisms advanced and the grounds on which they are based, will perhaps serve to show more clearly some of the difficulties involved and the precautions necessary in the use of the methods in this way.

In an attempt to determine whether respiratory gradients exist, Shearer (1924) stated that there are serious objections to the Winkler method but did not say what they are. Parker (1929, p. 422) agrees with Shearer but also fails to state what the objections are. In a later paper Shearer (1930, p. 264) says that the error resulting from the discharge of slime and tissue fluids into the water is always very great where a large number of animals is employed, but he presents no data to confirm his assertion or to show how great the error may be. In fact, there is no evidence in these papers to show that either Shearer or Parker has ever used the Winkler method. Needham (1931, p. 586) also questions the value of data obtained by Hyman with the Winkler method on the basis of a personal statement of opinion by Shearer, but again without presenting any evidence in support of his view. These somewhat dogmatic expressions of opinion without evidence to support them can scarcely be regarded as valid scientific criticism and are certainly an inadequate basis for discarding a large body of highly consistent positive data. Moreover, in the use of the Winkler method in connection with the gradient problem the relative, rather than the absolute, rates of oxygen consumption of similar pieces from different body-levels, or of the same pieces at different times, are the important data. Since equal, or approximately equal, numbers of pieces and equal test periods have been used in lots to be compared, any possible error due to slime production or other substances from the pieces should be approximately the same in different lots. Actually, however, pieces of *Dugesia dorotocephala* used in many determinations do not produce large quantities of slime when undisturbed in normal environment, as they are during respiratory periods for Winkler determinations; and any slime that is produced does not immediately spread through the water but remains on the wall of the container, or, in the case of small pieces which do not move about, it may form an envelope about the piece resembling a cyst, and the piece may remain in it for several days. Samples of water taken from the container for analysis contain little or none of this slime. Cut surfaces of planarian pieces, hydroids, and other forms contract rapidly after section, so that the discharge of tissue fluids occurs only during a short time. With planarian and hydroid pieces the contraction occurs before the pieces can be made ready for the respiratory period; moreover, determinations were made not only soon after section but also several hours later. Attention was particularly called to these points by Hyman (1916*b*) on first using the Winkler method, and determinations have been made repeatedly, showing that error from these sources is negligible (Hyman, 1932*a*). The criticisms of Shearer and Needham are without foundation. As regards the question of possible nitrite error in Winkler determina-

tions, a question raised in connection with other work with this method, Allee and Oesting (1934) have shown that Hyman's data on planarian pieces from different body-levels are not open to criticism. A recent comparison of the Winkler method with respirometer and Van Slyke methods has shown the Winkler method to be as accurate as the others (J. Wilder, 1937).

As a matter of fact, except for determinations with very small amounts of material, the Winkler method has certain very definite advantages over most respirometer methods, particularly with aquatic material easily excited to movement. The material is in normal environment and entirely undisturbed during the respiratory period, and motor activity is reduced to a minimum. With most respirometer methods the shaking and the starting and stopping tend to induce movement; and even if they do not induce visible movement, their effects on respiration are not known.

The assertion, unsupported by proof, was also made by Shearer (1930) that the differences in oxygen consumption found by Hyman in planarian pieces are too small to be significant; that they are statistically significant was shown by Hyman (1932a). The high degree of consistency of Hyman's data on pieces from different levels, on age differences, on effects of feeding and starvation, and on reconstitution in *Dugesia* with repeated determinations constitutes strong evidence that they represent real respiratory differences. Before they can be discarded, equally consistent and conclusive evidence must be presented to show that they are incorrect. Moreover, it is also of some interest to note that these differences in oxygen consumption are closely paralleled by differences in susceptibility to cyanide, and the differences at different body-levels, by dye-reduction gradients.

Averages of determinations of oxygen consumption by a respirometer method made by Shearer (1930) on two planarian species show a respiratory gradient; but, because of the great variations and because of observation of greater motor activity in anterior than in posterior pieces, the author holds the opinion that the respiratory gradient observed results from the differences in motor activity. Apparently he made no attempt to decrease motor activity, though it is possible, at least with most planarian species, to eliminate it almost entirely by using short pieces instead of large fractions of the body length. There is no evidence in his paper that the question whether a temporary increase of respiration followed section was considered, nor are there any data concerning the effect on respiration at different body-levels of presence or absence of food and digestive activity in the gut; it is not stated whether the heads of the anterior pieces were removed; motor activity is greater when the head is present. The data are not given in full but only as averages with a few of the determinations to show the variation. Determinations for the two species are not given separately, although the forms which he calls "*Planaria nigra*" and "*P. lactea*" (incorrectly) supposedly belong to different families and genera. The possibility that the great variation found may be due to the method and lack of proper precautions is not considered; and because of this variation, the consistency and uniformity of Hyman's data are criticized. He is apparently also ignorant of the fact that both Hyman and Parker used species with a posterior zooid (*D. dorotocephala* and *D. tigrina*), while the forms which he used, if they can be properly identified, have no such zooid. The posterior zooid has a respiration about as high as that of anterior regions of the anterior zooid.

Concerning determinations made on *Thysanozoon*, a polyclad, Shearer (p. 263)

makes the remarkable statement that the ratio of oxygen consumption in different pieces "was just about the same as the relative amount of movement of these parts during the course of the measurements." In a determination on anterior and posterior halves of *Thysanozoon* the pieces remained 48 hours in the respiratory chamber before determinations and had apparently come to rest, but "some movement invariably took place at long intervals." In this case the oxygen consumption of the posterior half was 26 per cent less than that of the anterior half. The question whether some movement at long intervals is sufficient to account for so great a difference is not considered. In view of the obvious lack of the precautions necessary in determinations of this sort, Shearer's data cannot be regarded as having any significance whatever, and certainly not as valid evidence against the highly consistent data which have been obtained in repeated experiments performed with proper care.

The method of comparative estimation of CO_2 production used with planarians and with *Corymorpha* has been criticized by Parker (1929) and by Needham (1931, p. 586) on the ground that precautions were not taken "to ensure that the acidity measured was due to carbon dioxide and not to other acids." Whether other nongaseous or nonvolatile acids or acid-producing substances than CO_2 are produced is easily determined by bubbling air through the indicator solution or by allowing it to stand exposed to air in a thin layer after change in color by, and removal of, animals or pieces. In one of the earlier papers concerned with this method attention was called to this point, and it was stated that with this procedure the color does return (Child, 1919b). It was not considered necessary to repeat this statement in later papers; but the test was consistently made, and consequently the criticisms are not justified. With this method the material is undisturbed during the respiratory period, but the method used by Parker involves continuous movement of the apparatus, and a small amount of water is desirable. With sluggish forms like *Stylochus* (Watanabe and Child, 1933) consistent results can be obtained. Parker says, regarding the planarian pieces used in his experiments: "Ordinarily they remained quietly in the bottom of the scoop," and, as regards *Nereis* pieces, that they "remained in relative quiescence." Here, as in the respirometer methods, the question of the effect of motor activity on respiration arises. There can be no doubt that with the Winkler method motor activity is less than with the other methods used in work of the kind under consideration; also, the use of a considerable number of pieces eliminates or decreases the effect of individual differences. At best, however, respiratory determinations on pieces from different body-levels of adult animals constitute only contributory evidence on the gradient problem, but their close agreement with other lines of evidence increases their value.

For further critical discussion see Watanabe and Child, 1933.

APPENDIX II

Methylene blue and Janus green have been found most useful in work on differential dye reduction. The former has been used both as an oxidized dye and as a leucobase, the latter in oxidized form. A considerable number of samples from different sources have given essentially similar results. With most forms tested, methylene blue is less toxic than other dyes used, except, perhaps, brilliant cresyl blue. Janus green has proved particularly useful because of the change in color from the blue green of the oxidized form to brilliant red with partial reduction. This makes it possible to distinguish slight differences and slight degrees of reduction more clearly than does the mere loss of color on reduction of methylene blue and various other dyes. With some forms the further reduction of Janus green to the colorless leucobase has been observed. In all samples used, Janus green has been found much more toxic than methylene blue, and great care has been necessary in its use in order to avoid alteration of the normal reduction pattern by injury of the more susceptible regions and consequent retardation of reduction or complete failure to reduce in them. Thionine and cresyl echtviolett have been used for comparative purposes with results similar to those obtained with Janus green and methylene blue. In fact, in every case thus far, reduction gradients have been the same with different dyes.

The procedure followed with small forms—protozoa, and embryonic and larval stages of echinoderms and other animals—consists in sealing a considerable number of individuals in a cell of small volume, usually made by drawing a ring of melted vaseline of the desired size on a slide and covering the material in water without air bubbles. The oxygen uptake of the animals brings about gradual oxygen decrease; consequently, reduction occurs gradually, and slight differences are distinguishable. With oxidized dyes the material is either stained before sealing or a concentration of dye determined by preliminary experiment to be nontoxic within the period preceding and during reduction is used as medium in the cell. With this latter procedure staining and oxygen decrease are occurring at the same time; and with certain concentrations of dye and rate of oxygen decrease, reduction may occur in one region while another is becoming more deeply stained. For elongated hydroids of considerable size, such as *Corymorpha*, elongated cells may be made; or for these and for earthworms and other large annelids glass tubes large enough to admit the animal may be used. Mineral oil has been used in some cases to exclude air. With many forms rapid staining with a relatively high concentration of oxidized dye and rapid reduction following have been found less likely to produce toxic effects than slow staining with a lower concentration. Toxic effect seems to depend on accumulation of the dye on certain cell constituents which appear as deeply stained granules, rather than on its mere presence in the cell. When the deeply stained granules become visible, reduction is retarded. With slow staining the granular accumulation may occur before reduction, and the reduction picture may be altered; with rapid staining and quick reduction this effect may be avoided. That this holds for all material is not asserted; it appears to hold for

embryonic and larval stages of echinoderms and for planarians and more or less transparent oligochetes. The method, properly used, is extremely delicate and of great value as a means of making directly visible certain characteristics of developmental pattern in intact embryos and larval stages of small size, as well as many larger developmental stages. It also serves to show an oxidation-reduction pattern in the ectoplasm of ciliate protozoa and in adult individuals of many metazoa. In order to avoid misleading results, however, it is necessary to use a wide range of concentrations and staining periods; only in this way is it possible to make certain that an observed differential or absence of a differential is not due to differential toxic action of the dye and consequent retardation of reduction in the injured regions (see pp. 67-70; also Child, 1936*a*, *b*).

APPENDIX III

The following papers are more or less directly concerned with differential susceptibility, chiefly with differential death and toxic effect. Material and agents used are given. Algae: Child, 1916*c*, *e*, 1917*a*, *b*, 1919*f*, KCN, ethyl alcohol, HgCl₂, CuSO₄, H ion (HCl), OH ion (KOH, NaOH), neutral red, KMnO₄, staling water, also unpublished data on a number of species exposed to ultra-violet radiation and to visible light with sensitization by eosin which show the same susceptibility gradients as with chemical agents. Protozoa: Hyman, 1917, *Amoeba*, KCN; Bovie and Barr, 1924, *Amoeba*, ultra-violet; Bills, 1924, *Paramecium*, alcohols; Child and Deviney, 1926, *Paramecium* and other ciliates, KCN, NH₄OH, NH₄Cl, NaOH, NaHCO₃, NaHCO₃+CO₂, CH₃COOH, HCl, H₂SO₄, neutral red, methylene blue, ultra-violet radiation, visible light after sensitization by eosin, lack of oxygen; Merton, 1929, *Vorticella*, formol, acetic acid, pilocarpine, alcohol; Monod, 1933, ciliates, ultra-violet. Coelentera: Child and Hyman, 1919, three species of hydra, KCN, ethyl alcohol, ethyl ether, neutral red, methylene blue, Janus green; Weimer, 1928, hydra, KCN; Hyman, 1920*b*, *Tubularia*, KCN, ethyl ether; Child, 1919*d*, hydroids, KCN, ethyl alcohol, ethyl ether, ethyl urethane, HCl, MgSO₄, LiCl, neutral red, methylene blue, 1925*a*, blastulae and planulae of hydromedusae, *Phialidium*, *Gonothyraca*, *Stomatoca*, KCN, HgCl₂, neutral red, methylene blue, 1926*a*, *Corymorpha*, KCN, ethyl alcohol, ethyl ether, ethyl urethane, chloretone, HCl, NaOH, NH₄OH, NH₄Cl, LiCl, strychnine sulphate, nicotine, caffein, neutral red, methylene blue, hypotonic sea water, sunlight after sensitization by eosin; E. J. Lund, 1931, *Obelia*, KCN. Ctenophora: Child, 1917*c*, KCN, 1933*a*, KCN, ethyl alcohol, ethyl ether, chloretone, HCl, NaOH, KMnO₄, neutral red, methylene blue. Platyhelminthes: various species of planarians; Child, 1913*b*, 1919*c*, 1919*e*, 1930, 1933*c*, KCN, alkaline, neutral, and acid solutions, ethyl alcohol, ethyl ether, chloretone, NH₄OH, hypotonic and hypertonic salt solutions, distilled water, lack of oxygen; Behre, 1918, KCN at different temperatures; J. W. MacArthur, 1920, acids and bases, 1921, dyes; Buchanan, 1923*b*, 1926*b*, effects of anesthetics on susceptibility to KCN, 1930, distilled water and hypertonic salt solutions; Buchanan and Levengood, 1939, serum antibodies; Sivičkis, 1923, KCN; Hinrichs, 1924*a*, caffein; Strandkov, 1934, 1937, X-rays; Wiercinski and Child, 1936; Wiercinski, 1939, supersonic vibrations; differential susceptibility of planarians to formalin, nicotine, strychnine sulphate, ultra-violet, and radium has also been determined and is similar to that with other agents; *Stenostomum*, Child, 1924*b*, Fig. 42, KCN, methylene blue; *Stylochus*, a polyclad, developmental stages and adults; Watanabe and Child, 1933, KCN, alkaline and neutral, chloretone, hypotonic and hypertonic solutions, methylene blue, α -naphthol. Annulata: Hyman, 1916*a*, microdrilous oligochetes, KCN; Child, 1917*d*, developmental stages of polychetes, KCN, HgCl₂, NaOH; Hyman and Galigher, 1921, *Lumbriculus*, KCN; Parker, 1929, *Nereis*, KCN; Castelnovo, 1932*a*, *Limnodrilus*, KCN; Kawaguti, 1932, *Branchiura*, methylene blue. Echinoderms: Child, 1915*a*, eggs and developmental stages of starfish, KCN, 1916*a*,

developmental stages of sea urchin, KCN; Galigher, 1921*a*, developmental stages of the sand dollar *Dendraster*, KCN, NH_4OH . Vertebrates: Hyman, 1921, teleost embryos, KCN, NH_4OH , 1926*b*, cyclostome embryos, NH_4OH , CH_3COOH ; Bellamy, 1919, Bellamy and Child, 1924, frog embryos, KCN, HgCl_2 , NH_4OH , ethyl alcohol, low temperature; Cannon, 1923, frog embryos, KCN, HgCl_2 ; Buchanan, 1926*c*, chick embryos, HCN; Hyman, 1927*a*, *b*, chick embryos and hearts, KCN, NH_4OH , NaOH; Hinrichs, 1927, chick embryos, ultra-violet. Two papers are comparative studies of susceptibility of animals from different groups to a series of related agents or to a single agent: J. W. MacArthur, 1921, susceptibility to basic and some acid dyes of *Paramecium*, *Dileptus*, *Hydra*, *Dugesia*, several rhabdocoels, and microdrilous oligochetes; Hinrichs, 1924*b*, ciliate protozoa, *Hydra*, *Dugesia*, microdrilous oligochetes, ultra-violet and visible light after sensitization by eosin. For general discussions of differential susceptibility see Child, 1914*f*, 1920*b*, 1923*b*, 1924*b*, pp. 76–80, 1928*d*; Hyman, 1926*b*, pp. 112–15; Watanabe and Child, 1933; Buchanan, 1930, 1935. Unpublished data on *Volvox* show the same death gradient with KCN, ethyl alcohol, HCl, hypertonic solutions, NaCl, sea water, ultra-violet, visible light with sensitization by eosin, and lack of oxygen in darkness. Numerous other publications concerned with action of external agents on living organisms give incidental evidence of differential susceptibility. This seems to be particularly the case with work on irradiation by ultra-violet, X-rays, and radium. Often, however, the use of only one or a few concentrations or intensities which are found to produce an effect, and of temporary exposure, make it difficult to determine whether, or to what extent, the results represent differential inhibition, differential tolerance, or differential recovery.

The work directly concerned with the problem of differential susceptibility began with the discovery of differences in survival time of physiologically young and old planarian individuals and of death gradients and tolerance gradients within the individuals, resulting from exposure to certain concentrations of ethyl alcohol and KCN (Child, 1911*e*; 1913*a*, *b*, *d*; 1914*b*). Further investigation indicated that these differences in susceptibility were related to real differences in physiological condition; and it began to appear probable that study of susceptibility to cyanide in different organisms, particularly in unicellular forms—forms consisting of a single cell series, such as many algae, eggs, embryos, and other small organisms—might afford data of interest and perhaps serve to indicate regional physiological differences which could not otherwise be determined in small organisms. In view of the fact that cyanides and KCN had been shown to be powerful inhibitors of many physiological oxidations, a general relation between the differences in susceptibility to cyanide and differences in rate of respiration or oxidation seemed highly probable. For this reason KCN was used extensively in the earlier experiments, with the result that an axial differential susceptibility, as indicated by the progress of cytolysis, disintegration, and death along the axis was found to occur in the forms examined, both plants and animals. In the attempt to throw more light on the problem data concerning respiratory metabolism and the effect of KCN upon it were obtained for different regions of the body and for individuals in different physiological condition, which had been found differently susceptible to KCN. The earliest respiratory data on planarians were comparative estimations of CO_2 production on pieces from different body-levels by means of the Tashiro biometer (Child, 1913*a*; Behre, 1918). The Tashiro apparatus has been super-

seded by other methods, but it should not be forgotten that it represented one of the pioneer methods in the field. Later CO₂ estimations were made colorimetrically (Child, 1919*b*, *c*; Robbins and Child, 1920). Differential susceptibility to lack of oxygen was found to parallel cyanide susceptibility in the forms tested. Comparison of the data appeared to indicate that differential susceptibility to KCN may serve as a rough indicator of quantitative differences in respiratory or oxidative metabolism in many organisms. The determinations by Hyman of the action of KCN on oxygen consumption (Hyman, 1916*b*; 1919*a*, *c*; 1920*c*) and on oxygen consumption in *Dugesia*, fed and starved individuals (1919*b*, 1920*a*), pieces and animals after reconstitution (1919*c*, 1923*b*), and young and old individuals (1919*d*) provided a more adequate basis for comparison of respiration and susceptibility to KCN and further evidence of parallel differentials and individual differences.

Some of the results of these investigations and the conclusions drawn from them aroused criticism of the hypothesis that differential susceptibility to cyanide might indicate differentials in respiratory or oxidative metabolism. Certain of these criticisms were due to misunderstanding. On the basis of his findings that oxygen consumption is not decreased by KCN in starved *Paramecium*, even in gradually lethal concentrations, E. J. Lund (1918*a*, *b*; 1921*a*) held that conclusions drawn from differential susceptibility were invalid, and B. L. Lund (1918) maintained that differences in survival time in KCN of *Paramecium* and *Didinium* individuals of different age and nutritive condition are due to differences in permeability. The criticisms are, in large part, misdirected because based on the assumption that Child and Hyman had maintained that the toxic action of KCN is exerted only on the ectoplasm of *Paramecium* and on the body wall only in planarians. No such view was ever advanced by these authors. They merely pointed out that the susceptibility gradient observed in *Paramecium* occurred only in the ectoplasm and that the gradient in planarians could be observed with certainty only in the body wall because the concentration of KCN which reached the internal organs and the time when a lethal concentration reached them might depend to some extent on the susceptibility and time of disintegration of the body wall. That the internal organs of planarians were susceptible was shown clearly enough by their complete disintegration and particularly by the much later disintegration of the digestive tract in starved, than in fed, animals, as compared with the body wall; but whether definite susceptibility gradients were present in the gut or other internal organs, and, if so, whether they were in the same direction as the gradient in the body wall, could not be determined with any certainty.

Lund's conclusion that KCN has little or no effect on respiration in *Paramecium* has been in part confirmed by later work on *Colpidium* (Pitts, 1931), in which a decrease of 25 per cent was observed, and on *Paramecium* (Shoup and Boykin, 1931; Gerard and Hyman, 1931), showing little effect. According to earlier unpublished data obtained by Hyman, which are mentioned here with her permission, a considerable decrease in oxygen consumption of *Paramecium* sometimes occurs in KCN, but thus far the factors which determine the occurrence or absence of decrease are not known. In connection with this question it is important to note that the cyanide death gradient in *Paramecium* and other ciliates appears only in the ectoplasm. This, of course, does not mean that the entoplasm is not susceptible to cyanide but only that it shows no definite gradient, as might be expected, since it is in continuous circulation. It seems

to be evident that there are oxidation systems in *Paramecium* little or not at all affected by cyanide, but the data on the death gradient in KCN and on the effect of KCN on respiration are not comparable, because the former concern the ectoplasm only, the latter the whole organism. It is still possible that cyanide may decrease respiration in the ectoplasm of *Paramecium*; but, since the ectoplasm is, to a considerable extent, differentiated into structural constituents, some of which may not respire actively, the total ectoplasmic respiration may be only a small fraction of the total respiration, and its decrease by cyanide may not be evident with the methods available. It is certain that the ectoplasmic gradient of differential dye reduction in low oxygen (Child, 1934b) is the same as the susceptibility gradient to cyanide and to lack of oxygen.

G. D. Allen (1919a), Hyman (1919c), and Buchanan (1926a) found that oxygen consumption in *Dugesia* is decreased by KCN, and Lund (1921b) showed that it is also decreased by decrease in oxygen concentration. Determinations of CO₂ production in pieces of *D. agilis* after certain starvation periods led Allen (1919b, 1920) to question the data on differential susceptibility in *D. dorotocephala* and the inferences drawn from them. It was shown by Hyman (1919b, 1920a), however, that with a longer starvation period than that in Allen's experiments the respiratory changes in *D. agilis* were similar to those in *D. dorotocephala*. It was also found by Hyman (1919c, d; 1923b) that the differences in oxygen consumption in relation to reconstitution, physiological age, and size and level of origin of piece were parallel to the observed differences in susceptibility. Comparative colorimetric CO₂ estimations showed differences in CO₂ production parallel, in general, to the differences in oxygen consumption found by Hyman; differential susceptibility to lack of oxygen was found to be parallel to the cyanide differential; and it was shown that KCN and lack of oxygen are additive as regards their effect on survival time (Child, 1919c). Robbins and Child (1920) showed that differences in CO₂ production, as colorimetrically estimated, paralleled the differences in oxygen consumption and susceptibility. The action of KCN on oxygen consumption in various other invertebrates was also investigated (Hyman, 1916b, 1919a, e, 1920c; Galigher, 1921b; Allee, 1923); in all cases there was a decrease with the higher concentrations, but in some forms a primary increase occurred with low concentrations.

In planarians, as in *Paramecium*, data on susceptibility and on respiration are not comparable in all respects. As noted above, observations on differential susceptibility concern the body wall because susceptibility of internal organs cannot be determined independently of that of the body wall. In well-fed animals the gut disintegrates as early as, or even earlier than, the body wall; but after long starvation, later than the body wall. This difference is suggestive of a considerable difference in physiological condition between the actively functioning and the long-starved gut. Respiratory determinations include gut and body wall and other internal organs. Pieces from different body-levels of well-fed planarians usually do not show any consistent evidence of a longitudinal respiratory gradient. In order to obtain evidence of this gradient, as well as in most other determinations of respiration, it has been found necessary to keep the animals without food for at least a week or two before work with them, in order that the digestive tract may be empty and quiescent. When starved animals are fed, respiration increases very greatly. This brief survey of the question of cyanide susceptibility in relation to respiration in *Paramecium* and planarians indicates that

with proper care differential susceptibility may be a valuable, though a rough and imperfect, means of indicating quantitative differences in physiological condition in naked, aquatic organisms. As will appear, particularly in chapter iv, there is a general parallelism not only between cyanide susceptibility and respiration but, as far as data are available, between susceptibility and quantitative differences indicated by other methods, the indophenol blue reaction, differential dye reduction in low oxygen, etc.

With further investigation the question of the physiological significance of cyanide susceptibility has become only one aspect of the problem of differential susceptibility—at least as far as physiological gradients are concerned—for it has been found that axial differential susceptibility to many other agents, both physical and chemical, in certain ranges of concentration or intensity show the same relation to the axis concerned as does cyanide susceptibility. Moreover, this relation appears not only in differential lethal action but in differential effect on development. In the light of these facts the problem of the physiological basis of differential susceptibility has become a much more general problem. It appears now as the problem of the nature of the factors in living protoplasms that are concerned in determining that differences in susceptibility along physiological axes in early developmental stages and in many of the simpler organisms throughout life are so largely nonspecific for so many different agents which act on protoplasms in different ways. Some consideration of this problem is undertaken in chapter iii.

APPENDIX IV

The general coincidence of susceptibility and dye-reduction gradients and the similarity of these gradients observed in related species raises certain questions concerning the apparent absence of a secondary acropetal susceptibility gradient in the basal region of the sea urchin *Arbacia* and the starfish *Asterias*. A re-examination of these forms as regards both susceptibility and dye reduction is desirable; but, lacking this, certain possibilities may be noted. The change in condition in the basal region may conceivably occur later in *Arbacia* and *Asterias* than in the other species studied, though this appears rather improbable in view of the general similarity in all, except as regards absence of mesenchyme formation preceding gastrulation in asteroids. The laboratory notes on which the earlier papers on susceptibility of *Arbacia* and *Asterias* (Child, 1915a, 1916a) were based give some evidence of increased susceptibility about the blastopore in both forms after gastrulation has begun. At the time the papers were written, this was thought to be associated, at least in *Asterias*, with contraction of the blastopore observed in this form. In the observations on differential death in *Arbacia* it was noted that in late blastulae cells were sometimes given off from the midbasal region, the region of primary mesenchyme; but the possible significance of this fact was not realized. Some of the differential modifications of *Arbacia* development by external agents give evidence that susceptibility of the entoderm does increase at some stage of development; but, since they result from exposure to the agent during the whole, or a considerable, period of development, it is not possible to determine from them at what stage the change occurs. Irregularities in differential death, that is, cytolysis of a cell or a few cells earlier than others at that body-level, are not infrequent and have been thought perhaps to indicate that these cells were at a more susceptible stage of the division cycle than those about them. The cytolysis of basal cells in *Arbacia* was assumed to be due to this or some other incidental factor. As regards the differential modifications of development, attention was directed chiefly to the modifications of external form and proportions; and while the condition and degree of entodermal development were recorded and drawn, the question of relative inhibition of entoderm and ectoderm received little consideration. Re-examination of these notes on the early studies of susceptibility indicates that attention was so sharply focused on the primary basipetal gradient and the developmental modifications resulting from it that the significance of evidences of a secondary acropetal gradient was not realized. They seem to be an excellent example of failure to grasp the meaning of what is actually seen and recorded because attention is directed elsewhere. It appears possible, however, that the change in condition in the basal region of *Arbacia* is less extreme than in *Strongylocentrotus*. The invaginated entoderm of *Arbacia* is larger in relation to size of the blastocoel, and its wall is thicker than in *Strongylocentrotus*; also, it does not undergo so much elongation in reaching the stomodeal region, and its cells do not change in shape so greatly during these stages as in the latter form. These differences may signify that the change in condition in the basal

region and the entoderm, perhaps also the mesenchyme, is more gradual and reaches full development at a later stage in *Arbacia* than in *Strongylocentrotus*. In any case, a reinvestigation of differential susceptibility and differential dye reduction in *Arbacia* is necessary for further light on these points.

Attention must also be called to the possibility, noted elsewhere (Child, 1936a, p. 449), that the dye-reduction picture exaggerates more or less the change in condition in the basal region preceding gastrulation. The greater thickness of the wall in the basal region of the late blastula may bring about a more rapid oxygen decrease, and consequently an earlier reduction in the cells of that region, than in the thinner ectoderm. If that occurs, the differential reduction picture does not represent correctly the difference in physiological condition of ectoderm and entoderm mesenchyme. However, this factor of thickness of wall and volume of cells does not account wholly, if at all, for the apparent change in condition in the basal region. It was noted in the text that reduction at all levels is most rapid at the inner ends or surfaces of the cells, that is, the parts bounding the blastocoel. As immigration of mesenchyme occurs, the cells which lie in the blastocoel reduce more rapidly than the inner surfaces of any other cells of the blastula, and even before invagination the inner surface of the entoderm reduces more rapidly than the inner surfaces of ectoderm cells, except perhaps those in the apical region. Since this was not the case in earlier stages, a real change in physiological condition of mesenchyme and entoderm has evidently occurred.

It is also an interesting question whether this change is induced in the entoderm by the mesenchyme or is independent of it. The evidence of a similar, though apparently less extreme, change in the basal region of the *Patiria* blastula in which no primary mesenchyme is formed shows that in that form it appears in the entoderm without induction.

Even though evidence of a change in condition in the basal region seems to be conclusive, it may perhaps still be questioned whether this region actually attains before gastrulation a higher oxidation-level than the apical region. If the dye-reduction picture does exaggerate the change, the level may not actually be higher. On the other hand, cytolysis and death by chemical agents may be relatively somewhat retarded in the basal region by the greater thickness of the cell wall. If there is such retardation, the real condition as regards these gradient patterns in the later blastula may be intermediate between that indicated by differential death and differential dye reduction. But whatever the final conclusions as regards degree and stage, there seems to be conclusive evidence that a change in condition does occur in the basal region, beginning in the later blastula or at gastrulation in *Strongylocentrotus*, *Dendraster*, and *Patiria* and probably at this stage or later in *Arbacia*, *Echinarachnius*, and *Asterias*. The form of the *Arbacia* gastrula suggests that the change in condition in the basal region, or at least in the entoderm, preceding gastrulation is not so great as in *Strongylocentrotus* and *Dendraster*. The apicobasal axis of the *Arbacia* gastrula is relatively shorter, its walls are thicker, and the archenteron remains thick walled during invagination, most of its elongation and decrease in thickness occurring later than in the other forms.

These conclusions do not exclude the possibility that the gradient pattern, either from the beginning of development or from a later stage, involves specifically different substances differently distributed, as Runnström and his co-workers maintain; but even if this is the case, differential susceptibility, which is not regionally specific with different agents, and differential dye reduction may be expected to indicate similar gradient directions, as they do very generally.

APPENDIX V

Runnström regards his experiments with potassium-free sea water (1925*a*) as indicating that basal and ventral regions lose potassium more rapidly than other parts in absence of external potassium and undergo more decrease in colloid dispersion. He concludes that they are more permeable to potassium. His criteria of a ventrodorsal differential effect of lack of potassium are apparently based largely on individual cases of somewhat modified development.

The polar susceptibility gradient to lack of potassium is, according to Runnström, acropetal in early cleavage. This is opposite in direction to the lethal gradients observed by Child with various agents and to the dye reduction gradient; but if his identification of ventral and dorsal regions is correct, the ventrodorsal susceptibility gradient to lack of potassium is in the same direction as the lethal gradients and the dye reduction gradient.

Differential susceptibility to absence of potassium may perhaps involve other factors than those considered by Runnström. For example, there may be axial differentials in distribution of potassium in the egg; or in its absence externally its internal distribution may be altered by physiological factors, or there may conceivably be a differential tolerance to loss of it and a differential recovery from its absence on return to normal sea water. Withdrawal of an essential element such as potassium may have effects very different from those resulting from addition to the medium of a toxic agent. A susceptibility gradient to lack of potassium, whether it depends on differential permeability or on some other factor in the protoplasm, may have little relation to the gradients indicated by nonspecific susceptibility to positive action of chemical and physical agents or by dye reduction, and the effect may be still further complicated by differential recovery after temporary exposure to potassium-free sea water, the procedure in Runnström's experiments. Further experiment with a wide range of potassium content, both above and below that of sea water and with different exposure periods, appears desirable.



APPENDIX VI

In his experiments on differential susceptibility with *Rana temporaria* as material, Cannon (1923) used only HgCl_2 , and the only concentration mentioned in his paper is $m/1,000$. According to his brief statement of results, disintegration showed no definite relation to axes or other particular features of development. Entirely aside from the question of differential susceptibility to different agents, it is highly improbable that regional differences in susceptibility to a particular toxic agent in a developing embryo can have no definite relation. In the light of the great body of definite positive evidence of such relation these negative results must at least raise the question whether the procedure employed is adequate, but it does not appear that this possibility was considered by Cannon. In a re-examination of the matter Bellamy and Child (1924) found it necessary to separate individual eggs from the mass, to remove the jelly, and to agitate the solution. If these precautions were not taken, differences in susceptibility might depend on differential exposure rather than on differences in physiological condition. In the use of HgCl_2 it must also be borne in mind that even rather low concentrations act as fixing or coagulating agents and kill without any cytolysis. Except for a surface effect on more advanced stages, apparently an action on the cilia or the ciliated cells, Bellamy and Child found differential susceptibility to HgCl_2 essentially similar to that observed with other agents.

Cannon also calls attention to the fact, previously noted by Bellamy, that alcohol produces changes in the basal yolk-laden region earlier than elsewhere. It was pointed out by Bellamy and Child that this is evidently an effect of a lipoid-soluble and lipoid-solvent agent on the yolk and has no relation to the differential susceptibility of the protoplasm. In the amphibian egg the yolk content of the basal region is so great that susceptibility of that region is more or less specific for fat-soluble agents.

The argument advanced by Cannon that the disintegrating tissue is toxic and that disintegration, once initiated at any point, will continue because of this toxicity is shown by many facts to be without foundation. First, disintegration may be arrested by return of the organism to the natural environment; and the parts which have not disintegrated may recover and develop further, or, as in planarians, undergo reconstitution. Second, when a localized area or gradient arises in some region other than the end of an axis, for example, in an organ primordium, it may remain localized or may progress in one direction and not in others, according to the pattern of the organ concerned. Third, in cases of partial differential tolerance with death and disintegration of certain regions cessation of disintegration and finally reconstitution of the parts which disintegrated may occur with continued exposure to the agent which originally produced the disintegration (see pp. 113-17).

In experiments on differential modification of cleavage by KCN Cannon found that in *Bufo* toxicity decreased, and in *Rana* increased, with decrease in concentration. The frog eggs were removed from the ovary and fertilized by addition of sperm, while the toad eggs were laid and fertilized naturally. The possibility that the higher con-

centrations of cyanide interfered with the swelling of the jelly in the frog eggs and decreased permeability in the membrane is the only basis that suggests itself for increasing toxicity of cyanide with decreasing concentration. This seems to be the only case known to physiology in which toxicity of KCN increases with decreasing concentration. In the case of the naturally laid and fertilized toad egg the membrane and jelly had attained their characteristic condition before the egg was subjected to cyanide. At any rate, it appears highly improbable that opposite relations as regards toxicity and concentration of KCN should exist in two so closely related protoplasms as those of frog and toad. That some factor external to the egg itself is probably responsible for the results with the frog seems certain.

APPENDIX VII

The following papers are concerned with planarian head frequency: *Dugesia dorotocephala*: Child, 1911*b*; Child and Watanabe, 1935*a*, length of piece, body-level, effect of delay of anterior and posterior section; Child, 1911*f*, 1920*a*, effect of nutritive conditions, size of animal (physiological age), stimulation; Child, 1916*b*, alteration of frequency by KCN; Behre, 1918, alteration by temperature; Buchanan, 1922, alteration by anesthetics; Hinrichs, 1924, alteration by caffeine; Rulon, 1936*a*, 1937, alteration by CO₂, H ion, organic acids, and calcium, effects of previous conditioning to CO₂ and to H ion; F. S. Miller, 1937, alteration by strychnine. Other species: *D. tigrina* (= *maculata*): Watanabe, 1935*b*, length of piece, body-level, effect of delay of anterior and posterior section and section of nerve cords; *Planaria lata*, now also regarded as *D. tigrina*: Sivičkis, 1923, length of piece and body-level; *P. gonocéphala*: Abeloos, 1928, length of piece and body-level; *Phagocata gracilis*: Buchanan, 1933.

In a lot of pieces as nearly alike as possible as regards length, level of origin, size and physiological condition of parent animals, and reconstititional environment there may or may not be uniformity of head form, according to conditions. In pieces representing a large fraction of body length and developing in normal environment the uniformity is high—almost or quite 100 per cent normal heads. In pieces representing very small fractions of body length in normal environment the uniformity is also high—almost or quite 100 per cent acephalic. In lots intermediate between these extremes and in pieces subjected to experimental conditions which alter head frequencies, a certain range of head forms usually appears in percentages characteristic for the physiological and environmental conditions. Such differences undoubtedly result in part from slight differences in physiological condition and susceptibility of different individuals and in part from unavoidable variations in length of pieces and level of origin. Frequencies of the different head forms can be tabulated numerically or as percentages, and a general comparison made from these data; but for graphic presentation and comparison it is convenient to have a value for the head frequency of a lot rather than the individual frequencies. Such values, adequate for the comparative purposes for which they are used, are readily obtained as follows: numerical values are assigned to each of the head forms—for example, normal, 100; teratophthalmic, 80; teratomorphic, 60; anophthalmic, 40; acephalic, 20. From these values an index for the lot is obtained by multiplying the number of cases of each head form by the numerical value for that form and dividing the sum of these products by the number of pieces in the lot; or, if the frequencies are expressed in percentages, multiplication of the percentages by the numerical values and division of the sum of the products by 100 gives, of course, the same result. The procedure is clear from the formula

$$I = \frac{100n_5 + 80n_4 + 60n_3 + 40n_2 + 20n_1}{N},$$

in which n_5-n_1 are the numbers or percentages of the different head forms (n_5 , normal, to n_1 , acephalic) and N is the total number of pieces in the lot or of living pieces, or, in percentages, 100. Since it is not certain that the different groups of head forms represent corresponding amounts of physiological difference or change, the values assigned are arbitrary, except that they indicate the order of degree of differential inhibition. The value, I , therefore is not a true mean head frequency but merely an index.

The recent application by W. A. Castle (1940, "Methods for evaluation of head types in planarians," *Physiol. Zool.*, 13) is of particular interest as providing a basis for more accurate general expression of head frequency data. However, the head frequency curves obtained in this way differ little in most cases from those graphed from the "head frequency indices" obtained by the method described above. The following quotation from Castle's paper summarizes the situation:

It is not to be inferred that the originators and users of the old scale of indices intended it to be more than an approximation for convenience. Indeed, Buchanan has stated [1923a, p. 410] that "no claim is made that the values 5, 4, 3, 2, 1, represent any exact mathematical valuation of regenerated tissue; this is simply a convenient and consistent way of showing the effects of the agent on head frequency." For the purpose designed, the old scale of equal intervals appears in most cases to have been entirely adequate for outlining major differences in head frequency along the axis of the planarian body or for distinguishing between the head frequencies of experimental and control groups where the differences are not obscure. Where such differences are slight, however, the use of a scale in which the indices or class ranges do closely approach the values based on observed frequencies of occurrence of the regenerated structures probably comes closer to revealing the real relationships between control and experimental groups.

APPENDIX VIII

Most of the experimental modifications of echinoderm development recorded elsewhere have resulted from action of single or a few concentrations or intensities of action of external agents. Many are effects of artificial sea waters differing from natural sea water in absence or increase in amount of certain components or substitution of certain components by others not present or present only in very small amounts in natural sea water. In spite of the great volume of experiment on echinoderm development there have been relatively few attempts to determine for single agents the effects of a wide range of concentrations, intensities, and exposure periods. It seems often to have been taken for granted that the modification produced by a particular concentration or intensity is specific. Also, the possibility of secondary modifications resulting from differential tolerance, conditioning, or recovery, and opposite in direction as regards form and proportions from primary differential inhibitions, has been largely ignored. In view of this situation a few further data concerning effective ranges of concentrations, exposure periods, etc., supplementing those in the figure legends, are given here.

With any agent in any effective concentration or intensity a considerable range of modifications occurs. These depend in part on differences in susceptibility of individual eggs and eggs of different females, but difference in conditions to which individuals are subjected at different stages of development in the container may also be a factor. Animals remaining on the bottom instead of swimming, and particularly aggregations, if allowed to persist, show more extreme differential inhibition with certain agents (e.g., KCN, LiCl) than animals swimming free. On the other hand, concentration of an inhibiting agent may become lower about an aggregation of animals because the agent is taken up by the cells. If there is a free surface exposed to air, animals reaching that surface may be much less inhibited than those that do not reach it. These effects of local differences in conditions in the container can be decreased by frequent gentle agitation, by decreasing surface exposed to air, and by use of large volume of medium. Undoubtedly, very slight physiological or environmental differences may determine whether the final result in a particular individual is a modification representing differential inhibition or one with some degree of differential tolerance, conditioning, or recovery. It is true, however, that differential inhibition is universal or predominant with higher concentrations or intensities and longer exposure periods; with decrease in concentration differential tolerance and conditioning become increasingly evident; and with certain ranges of concentration or intensity the modifications of differential recovery become predominant following return to water. Degrees of secondary modification differ widely with different agents: with KCN, for example, differential tolerance or conditioning is very slight or absent in the brief period of development, but more or less differential recovery may occur after return to water from low concentrations. With LiCl the secondary modifications occur to a much greater degree; with alcohol and various other agents they occur rapidly and to extreme degrees. The differential in-

effect at different levels of a gradient also differs with agent. With KCN it is relatively slight; with CuSO_4 and HgCl_2 , considerable, at least for *Arbacia*; with LiCl, relatively great for all forms used. These differences are doubtless related to the particular way in which a given agent brings about its effect.

KCN has been used in many concentrations from m/100 with brief temporary exposures to m/200,000, m/300,000, and m/400,000 with continuous exposure. Ethyl alcohol, 3-4 per cent, is gradually lethal for *Arbacia*, but with temporary exposure differential inhibition is followed by rapid and extreme differential recovery; differential tolerance and conditioning following inhibition appear in 2 and 1 per cent (*Arbacia*). Rapid and extreme secondary modifications follow the initial inhibition in sea water to which certain concentrations of acetic or hydrochloric acid are added, the effective agent undoubtedly being CO_2 set free by the acid. In earlier experiments with *Arbacia* the pH was not determined, but it was determined later that the concentrations of acid added were not high enough to make H-ion concentration effective. Sodium hydroxide from m/1,000 to m/5,000 inhibits differentially, but with secondary conditioning in the lower concentrations and recovery in water (*Arbacia*). Primary differential inhibition and secondary modifications occur in hypotonic sea water (90 and 75 per cent. CuSO_4 from m/100,000 to m/2,500,000 inhibits differentially; rather extreme forms of differential recovery follow returns to water from even the higher concentrations; and with continuous exposure to the lower concentrations forms showing marked degrees of differential tolerance or conditioning appear. HgCl_2 m/1,000,000 is strongly inhibiting differentially and finally lethal for *Arbacia*; in m/5,000,000 and m/10,000,000 there is primary differential inhibition with slight secondary modification in the higher concentration in 10-20 per cent of the culture, in the lower concentration in 80-90 per cent, forms with large oral lobe and wide brachial angle resulting.

Lithium chloride has been used in a large number of concentrations from m/200 to m/10 and with exposure periods ranging from 1 or 2 hours to continuous throughout development and beginning at different developmental stages; experiments have been repeated again and again with different lots of eggs, with the same concentrations and exposures and with slight variations. *Arbacia punctulata*, *Strongylocentrotus purpuratus*, *S. franciscanus*, *Echinarachnius parma*, *Dendraster excentricus*, *Asterias forbesii*, and *Patiria miniata* have all served as material for LiCl experiments. The special interest in this agent results primarily from its high effectiveness in producing exogastrulation; this has led many to regard it as regionally specific in action. In the writer's experiments LiCl has been used not only in the analysis of exogastrulation but for production of other modifications. With many concentrations effects of approximately isotonic solutions and of sea-water solutions which are somewhat hypertonic have been compared on the same material. In the high concentrations, which sooner or later stop development or are lethal, the hypertonic solutions are somewhat more effective in modifying development differentially; with temporary exposure they usually give a higher percentage or more extreme type of exogastrulation than the isotonic solutions; with lower concentrations no distinct difference in effect has been observed.

According to MacArthur (1924), m/160 is about the optimum for exogastrulation in *Echinarachnius*. Higher concentrations are required for *Dendraster*; 100 per cent or

nearly of exogastrulae usually results from 16 to 18 hours' exposure to m/50, m/40, or m/30 from the two-cell stage. Lower percentages occur with lower concentrations, increasing frequency of dissociation of the entodermal region, cessation of development and death, with higher concentrations. The higher the concentration with a certain exposure period the farther apically entodermization extends and the more frequent is dissociation of the original prospective entoderm. *Strongylocentrotus franciscanus* and *S. purpuratus* are apparently somewhat less susceptible to LiCl than *Dendraster*, and the first seems to be slightly less susceptible than the second.

Patiria is less susceptible than the echinoids. In m/30 for 14 hours or longer from early cleavage only 5-10 per cent exogastrulae appear: in m/20 for 18-25 hours from early cleavage 80-90 per cent are exogastrulae. With longer exposure to these concentrations more or less dissociation of the original prospective entoderm occurs, as in echinoids. With exposure beginning in later stages the frequency of exogastrulation and degree of entodermization decrease. Inhibition and more or less dissociation of invaginating entoderm, particularly of the enlarging apical region, occurs in m/40 with continuous exposure.

With concentrations of LiCl which produce exogastrulation in *Patiria* there is usually little differentiation of ectoderm, but with lower concentrations the same differential modifications of form and proportion appear as with other agents, primarily differential inhibition, secondarily the opposed modifications of differential tolerance, conditioning, or recovery. Extensive entodermization of prospective ectoderm has been obtained in *Patiria* in Janus green 1/2,000,000. In the writer's experiments the susceptibility of *Asterias forbesii* was about the same as that of *Patiria*, but MacArthur has reported exogastrulation in that species and in *Orthasterias* with LiCl m/100 to m/160.

Differential inhibition from crowding probably results from lack of oxygen or from some toxic metabolite rather than from CO₂, since it may be extreme with pH no lower than 7.3-7.4. Concentrations of the dyes used to produce differential modification have little significance, provided they are low enough, for they are accumulated within the cells even from extremely low concentrations in the water.

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